ANATOMICAL AND MECHANICAL CHARACTERISTICS OF WOODS USED TO MANUFACTURE BASSOONS

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By

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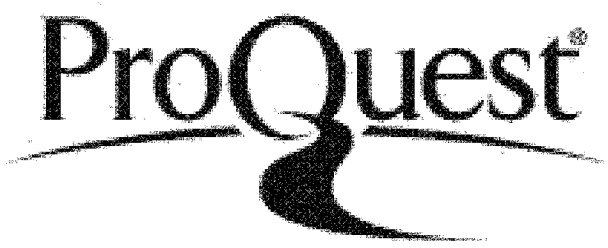

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Abstract

The purpose of this dissertation was three-fold – 1) to determine if anatomical characteristics and mechanical characteristics derived from tapping (the act of striking an object lightly) can be used to more accurately describe bassoon resonant wood than the characters in use now, 2) to determine if any Alaska hardwoods can be used to construct bassoons, and 3) to produce lists of potential North American hardwoods and resonant bassoon wood characters. The bassoon resonant woods (*Acer* spp., *Dalbergia melanoxylon*, and *Pyrus* spp.) were compared to a known non-resonant bassoon wood (*Juglans nigra*). Vessel length and width, fiber length, and axial parenchyma width were measured in sectioned and macerated wood slides, along with the ratios of crystalline cellulose, lignin, pectin, and other aromatics in the cell wall. Partial frequencies created from tapping specimens on each longitudinal face were measured from melodic and peak partial spectrograms, as well as the spectrum obtained from the beginning of the sound. MANOVA and univariate ANOVA showed the resonant woods were significantly different from the non-resonant *Juglans nigra* using the characters measured. These characters were then used to compare two Alaska hardwoods (*Alnus rubra* and *Betula neoalaskana*) to the temperate resonant woods (*Acer* spp. and *Pyrus* spp.) and the non-resonant *Juglans nigra* using k-means clustering, MANOVA, and univariate ANOVA. Both Alaska hardwoods grouped with the non-resonant *Juglans nigra.* Lastly a list of potential North American hardwoods to be checked anatomically was compiled, as well as a list of characters that combine those used now as well as characters found in this study.

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CHAPTER 1: OVERVIEW – THE BASSOON, WOOD ANATOMY, VIBRATION & SOUND, AND THE POTENTIAL OF ALASKA FORESTS

The purposes of my study are: 1) to determine if characters derived from wood anatomy and mechanical characters derived from tapping can be used to better describe bassoon resonant woods than the characters in use now, 2) to determine if the Alaska hardwoods can be used as an alternative material for the bassoon, and 3) to provide lists of potential species and characters to describe bassoon resonant wood. Some of the characteristics used to describe bassoon resonance wood – clear, defect-free wood that dries straight and can be worked easily with a lathe – have been developed over the last 300 years. The other characters– density and elasticity – are based on research done with the violin. Through experiments by manufacturers, it has been shown that these characteristics do not work to accurately determine bassoon resonance wood. To find these descriptive features of bassoon resonance wood, the bassoon's construction and history, the basics of wood anatomy, processing, vibration, and sound, need to be understood.

The Bassoon

The bassoon is the bass member of the woodwind family. It is essentially a nine foot conical tube folded almost in half. The bassoon body comes in four parts, also known as joints: the bell, the long joint, the boot, and the wing. Along with the body, there is also a bocal, a metal crook-shaped tube that continues the conical tube (Figure 1), and a double reed (Langwill 1971b). The bassoon is a relatively primitive instrument, and its history not only explains its final shape but also why the materials used to make the bassoon are chosen and how sensitive the instrument's unique sound is to change.

The basic configuration of the bassoon comes from four instruments – the flute, shawm, racket, and the dulcian. The bassoon is a modification of the six tone-hole system. The flute introduced this system to music, and all ancient and extant woodwinds are variations (Baines 1991). Air within the instrument is vibrated in some way and circulates around, some of it escaping either through the tone holes or the bell (the end of the instrument) (Benade 1976). Also, bassoons have conical bores, meaning the interior of the instrument tapers from top to bottom. The shawm was the first instrument with this bore type and a double reed, making it the precursor to all double reed instruments. The double reed brought a more complex and louder sound that could be unstable tonally; the conical bore fixed the tone by stabilizing it to a certain extent (Montagu

1976). The rackett and dulcian introduced the folded bore configuration and bocal that characterizes the bassoon, making the instrument easier to play (Kilbey 2002, Waterhouse 1984a). With these introductions, the bassoon that we recognize today came into existence.

No one knows exactly who developed the bassoon or when it happened. The word 'basson' starts appearing in scores and one of the composers known for using both the bassoon and oboe came to prominence during the beginning the 17th century. The composer Jean-Baptiste de Lully moved to France and came into the sphere of influence of a family of instrument makers living near Paris, the Hotteterres. Composers considered the double reeds to be the embarrassing relatives of the more popular stringed instruments before Lully. They had severe intonation (tuning) problems and limited note range. The Hotteterres were important, because they introduced the concept of the 'jointed' instrument, an instrument comprised of smaller sections that form a larger, contiguous instrument when put together. This jointing concept improved intonation by enabling the bores of the bassoon to be drilled with more precision (Marcuse 1975, Waterhouse 1984b). The new jointed design stabilized intonation and expanded the range of the instrument, making it easier for composers like Lully to incorporate it into their compositions. More keys were added to accommodate composers' demands for a greater range of tones over time, culminating in the classical bassoon. The classical bassoon had a four octave range and a unique voice, called the *vox humana*. Its tonal stability was better than its predecessors, and it was easier to play. However, the intonation was still unstable compared to the string instruments and the flute (Langwill 1971a, Waterhouse 1984b, Zerkle 1942).

During the 19th century, the evolution of the bassoon split along two paths – the French and German systems. Each had the overall characteristic bassoon sound, but they differed in subtle ways. The French system kept the classical bassoon intonation and projection, whereas the German system had intonation qualities comparable to the dulcian and blended with the orchestra instead of projecting over it (Baines 1991, Pitt 1978). The French system of the bassoon evolved slowly from the classical bassoon through a series of musician-craftsmen partnerships. All the changes were subtle to preserve the unique sound of the classical bassoon. Jean Nicolas Savary, a French bassoonist and instrument maker, may have been the first to start the modifications, and the major changes ended with Eugène Jancourt, a French composer and bassoonist. The system added keys, stabilized many of the notes, and increased the playing range beyond that of the classical bassoon without sacrificing the unique tonal character. The wood used to make the

instrument was also changed from the traditional European maple and pear to the tropical African blackwood (*Dalbergia melanoxylon*) (Pitt 1978). This change is probably due to France's colonization of many parts of Africa during this time, making this wood available for export (Suret-Canale 1971). The German system evolved from a collaboration between bassoonist Karl Almenräder and manufacturer Johann Heckel, based on the work of physicist Gottfried Weber. Almenräder completely redesigned the instrument after reading Weber's works on musical acoustics (Langwill 1971a). According to one source (Baines 1991) this drastic redesign obliterated the characteristic bassoon sound, and Heckel had to step in and redesign the instrument more to bring back some of its characteristics. The final system had fewer keys than its French counterpart, it was easier to play than the French system, and the redesign stabilized the lowest register. The altered system also produced an instrument that was able to blend with almost any other orchestral instrument and had stable intonation (Waterhouse 1984b, Zerkle 1942).

Three changes occurring in the 19th and 20th centuries demonstrate how important the material, the reed, and the design of the instrument are to the characteristic bassoon sound. During the 19th century the Boehm system, a system of fingering and keywork developed by the flautist Theobald Boehm, revolutionized both the flute and clarinet by creating a logical fingering system. Multiple instrument makers tried implementing the Boehm system for the bassoon, and all were unsuccessful. Somehow the Boehm system made fingerings harder, and worse, the instrument lost its characteristic sound. No later alteration could bring the bassoon sound back, so the Boehm system for the bassoon was abandoned in both the German and French systems (Waterhouse 2003). The second change occurred at the end of the 17th century. The woodwinds did not project well outside, and other instruments were developed from various metals, mostly brass, to replace them. Most successful was the saxophone, which replaced the single reed instruments. Less successful was the sarrusophone, which was designed to replace the double reeds. Because metal is heavier than wood at equal thicknesses, the bassoon's design had to be altered drastically. Unfortunately the redesign reduced the tonal range of the instrument, the bassoon timbre was lost, and the instrument was difficult to play (Blaikley and Baines 1984). The final change was the creation/design of a single reed mouthpiece created to allow saxophonists to play the bassoon without learning how to use a double reed. Many of the overtones characteristic of the rich complex sound of the bassoon were lost with the mouthpiece (Spencer and Mueller 1986).

Bassoons have a long evolutionary path. While instrument designs, reeds, and even perceptions changed during this evolution, the material from which the bassoon was constructed, hardwood (a generic name for any woody angiosperm) remained constant. Either pear (*Pyrus communis*) or maple (*Acer* spp.) was used. Both were beautiful woods, easy to turn on a lathe, and affordable (Zadro 1975a, b). By modern times, North American and European maples were used to make German system bassoons. The French went a step further and moved to the tropical hardwood African blackwood (*Dalbergia melanoxylon*), which was equally beautiful but expensive (Langwill 1971b). The wood plays a role in the bassoon's sound, although the importance of the role is still being debated.

Wood for the bassoon is chosen with characters discovered over the centuries spent constructing the bassoon and its ancestors, along with characters discovered from research done on the violin. The most desirable wood is defect-free and clear, with straight or nearly straight growth rings and does not twist when the wood is drying. Research done on the violin revealed that density and elasticity were also found to be descriptive characters. In the case of woodwinds, the most desirable woods have at least a medium density and elasticity. After the wood is chosen, the timber (harvested wood) is seasoned for at least two years. The primary and secondary bores for the instrument joint are drilled, and the outside is shaped in stages. During each stage, the newly formed joint is tested to see if any dampening (muting of sound) has occurred during the shaping process. If dampening has started to occur, the joint is discarded and another is started. However, if it remains resonant throughout, the finished joint is dipped in oil to make it impermeable to water, stained, and finished.

Resonance is at the heart of two problems encountered when constructing bassoons – growth ring curvature and choosing wood based on characters currently used for resonance wood identification. If the turning blank used has growth rings that are too curved, dampening can occur during the shaping process. Not all turning blanks used for bassoons have perfectly straight growth rings, because the largest diameter trees that produce turning blanks with straight growth rings have already been harvested. Blanks with curving growth rings tend to dampen sound when shaped, but not all will do so. The line between too much curvature and just enough not to interfere with sound production has yet to be studied in bassoons. A similar study was conducted on arch angles in violin soundboards. A German luthier examined the dampening effects of arch angles in violin soundboards by measuring dampening in different arch angles. He found that

steep arches created a dampening effect on sound more than a shallow arch in carved wood (Schleske 1990). If drilling a bore through wood is equivalent to carving and a curving growth ring equivalent to the arch angle, this study would explain the dampening phenomenon seen with certain turning blanks containing curved growth rings. It also indicates that dampening could be affected by the proportion of cell wall layer exposed.

The other problem, choosing wood based on characters currently used for resonance wood, can be a time-consuming and costly proposition. Fox Products Corporation produced a bassoon made from black walnut (*Juglans nigra*), a wood that is similar to maple in matters of density, elasticity, and workability. The resulting instrument played well in the workroom, but its sound did not project well in a concert hall setting (Owen, *pers. comm.*), which is problematic, because bassoons are designed to be played in concert halls. This result runs contrary to the seminal work done on woodwind material, "Effect of wall material on the steady-state tone quality of woodwind instruments" by John Backus (1964). He attached a vacuum cleaner to the mouthpiece of clarinets made from three different materials – wood, plastic, and metal – and placed microphones over the tone hole joints. He then recorded the vibrations that resulted from turning the vacuum cleaner on. He found that while the wall material did vibrate, it was not significant enough to affect the timbre of the instrument. However, the experience at Fox does parallel a study done on the marimba. Brancheriau *et al.* (2006) compared a marimba maker's assessment of potential bar wood to anatomical and mechanical measurements to see if there was a correlation between the maker's perception and the characteristics attributed to these woods. They found the marimba maker's assessment of which woods were unsuitable corresponded with anatomical differences between the preferred and unsuitable woods.

Wood Anatomy

The preferred material for many instruments, including the bassoon, is wood (Baines 1969). Wood is a heterogeneous, orthotropic solid with three distinct sides. The sides, also known as faces, correspond to the tree's axes of growth; the transverse face (Figure 2a.) is parallel to the tree's girth growth (horizontal axis) and perpendicular to the vertical axis of growth; the tangential face (Figure 2b.) is perpendicular to the tree's girth growth and parallel to the vertical axis of growth; and the radial face (Figure 2c.) is parallel to both the tree's girth growth and vertical axis (Hoadley 1990). The preferred face for stringed and percussion instruments is the

radial face (Hurd 2004) and is presented to the instrument maker as quartersawn wood. Quartersawn is a timber cut oriented along the rays and is dimensionally stable (Hoadley 2000). Dimensional stability is dependent on the anatomy of the wood itself.

Wood is also defined as secondary xylem – the water conducting and structural system of all trees and shrubs. Secondary xylem (Figure 3) comes in two forms, gymnosperm (*i.e.* pine, spruce, fir) and angiosperm (*i.e.* oak, maple, sycamore). Gymnosperms, also known as the softwoods, contain various forms of tracheids and parenchyma. Tracheids (Figure 3c.) are xylem elements that run parallel with the vertical growth axis of the tree, transport water and nutrients from root to canopy, and provide structural support. They are produced in three forms – the traditional water conducting tracheid, the structural fiber, and the ray tracheid – a transporter of nutrients and water in the lateral direction (Esau 1977). Parenchyma (Figure 3d1.-d2.) grow mostly perpendicular to the vertical growth axis and transports and stores metabolic products (Dickison 2000). Another, less common, kind of parenchyma that grow parallel to the vertical axis of trunk growth, called axial parenchyma, acts as storage for metabolic products and various kinds of crystals. Angiosperms, also known as the hardwoods, contain vessels (Figure 3b.), fibers (Figure 3a.), and parenchyma (Figure 3d1.-d2.). The vessels and fibers divide the responsibilities of the gymnosperm's tracheids – the vessels transports water and nutrients and the fibers provide structural support. The parenchyma perform the same function as in the gymnosperms (Esau 1977). In the case of secondary xylem, function defines the form of the cell, specifically the cell wall.

Besides their functional differences, the xylem elements of wood have cell walls (Figure 4) that are created to accommodate their function. All cell walls contain four chemical components – cellulose, hemicellulose, lignin, and pectin (Mauseth 1988). Cellulose microfibrils and hemicellulose are woven into a framework in pectin produced by the Golgi apparatus (Dickison 2000), an organelle that synthesizes macromolecules in the eukaryotic cell (Dashek and Harrison 2006). Hemicellulose helps stabilize the cell during expansion (Mauseth 1988). Lignin is laid down to provide compression strength, antimicrobial protection, and hydrophobic properties to the cell wall after expansion is complete (Donaldson 2001). Primary xylem, such as that in herbaceous plants, contains only one wall layer. The secondary xylem in woody plants contains multiple layers. Along with the primary cell wall, it has a secondary wall that is laid down in two layers (Esau 1977). The amount of cellulose also increases in the secondary wall, because cells

with secondary walls typically have a more structural function. In the secondary wall, the first layer (S1) is generally thin and the second layer (S2) is much thicker (Dickison 2000). Of the two, the S2 layer influences the mechanical properties of the secondary xylem. Sometimes a thin third layer is produced, called S3 or the tertiary wall layer (Esau 1977). The structure of the cell wall is important to understand, because it ties directly to the mechanical properties of the wood.

How a cell wall reacts to an external force, like gravity or vibration, is directly linked to the mechanical properties of the chemical components, specifically cellulose, hemicellulose, and lignin. Cellulose has a high elasticity when measured with nanoindentation – a method of measuring elasticity at the microscopic scale (Salmén 2004), which means it takes a lot of energy to deform it. Lignin and hemicellulose have much lower elasticities (Salmén 2004), and it does not take much energy to deform it. As a result both lignin and hemicellulose become a sink for energy, absorbing the energy instead of reflecting it back (Dinwoodie 2000). Therefore, the thicker secondary walls will have a higher elasticity because of a greater amount of cellulose and be resistant to deformity. It can be extrapolated from this idea that cells with thicker cell walls have higher elasticities, and woods with thick cells also have higher elasticities. These higher elasticity woods tend to be denser, and density is one of the characters instrument makers consider when selecting wood for their instruments (Fletcher and Rossing 1998).

Vibration and Sound

The sound a bassoon makes is produced by vibration (Benade 1990). Vibration occurs when a solid or a medium is forced from a state of rest by energy and oscillates – moving back and forth – instead of in a straight line. Sound is produced when air is forced to vibrate in frequencies people can hear. Frequency is the number of oscillations per unit of time for music and is measured in hertz (Basu 2000). For a bassoon, the energy for sound is produced by the bassoon player blowing into a double reed (Benade 1990). The double reed is constructed from a piece of reed cane, *Arundo donax*, forming tapering sides, called blades, that enclose a cavity (Langwill 1971b). The reed's two blades oscillate, and the energy created by the oscillations is transferred to the column of air contained within the bassoon through the cavity. The column of air is located in the bore – the hollow part – of the instrument (Benade 1990), which is a hole produced by drilling. In the case of the bassoon, the main bore is parallel to the length of the instrument with smaller bores connecting the tone holes to the main bore (Langwill 1971b). The column of

air rotates inside the bore of the instrument until an opening is found for the air to escape, such as a tone hole being opened or through the opening in the bell. Opening the tone holes changes the length of the bore of the instrument, which determines how high the sound being produced will be (Benade 1990). The sound produced can be described quantitatively and qualitatively.

Quantitatively the bassoon's sound is described as a complex wave (Benade 1990). When sound waves are first taught in physics, they are drawn out as sine waves. The sine wave demonstrates the oscillation that typifies the motion created by a vibration. It also shows the amplitude, which correlates to loudness, and oscillation length (cycle), which correlates to the frequency (Avison 1989). Simple waves exemplify the sine wave, because they have no noise component to muddy the sound. Bells produce pure sounds. The sound is produced by a clapper striking the wall of the bell. Complex sounds are a conglomeration of partial waves. The partial sounds combine together to produce a new note. Some partials are higher than the combined note and some lower (Benade 1990). All wind instruments produce complex sounds, because the vibration is not initiated by the instrument body. In the reed woodwinds, the vibration is started by the reed; in the flute, the vibration is started by blowing air through a hole in the mouthpiece by the flautist; and in the brass instruments, the vibration is started by the player's lips (Fletcher and Rossing 1998).

The qualitative components of sound include timbre and tone color. A bassoon's signature sound, the sound that is easily recognizable as a bassoon, is called timbre (Holt 1990). Timbre is the result of both the vibration source and the design of the instrument (Moore 1995). Musicians describe sound using color terms, such as warm vs. cold and bright vs. dark (Holt 1990). These qualities are not really related to timbre. Bassoons can be warm or cold, depending on the room where the instrument is being played or the qualities of the reed being used. The signature of the bassoon's sound does not vary. Instead, these terms tend to be easier to hear in a more relative sense, that of the interrelated dynamic of a musical group. Warm sounds tend to blend in within a group, and cold sounds tend to float over the group. Bright sounds are more strident and do not blend in with other instruments. Dark sounds are more mellow and blend in well with other instruments (Holt 1990).

Like timbre and tone color, the interaction of waves produced also characterizes sound. Waves can potentially amplify or dampen each other. If the waves produced by an instrument amplify

each other, the amplitude and loudness of the sound increases. Dampening waves cancel each other out, causing the amplitude and loudness of the sound to decrease (Benade 1990). This is particularly important when dealing with resonance frequencies of materials. Resonance frequency is the maximum frequency at which a material vibrates when exposed to an energy source. When there is no dampening, the resonance frequency is equivalent to the natural frequency – the frequency at which a material will vibrate unforced. Some materials have a range of resonance frequencies (Fletcher and Rossing 1998). For example, if the resonance frequencies of the other instrument being played or the instrument body itself amplifies the frequency of the note being played, then the note becomes louder. If the resonance frequencies of the other instrument being played or the instrument body itself dampens the frequency of the note being played, then the note gets softer (also known as muting). All of these qualities of sound contribute to the uniqueness of the bassoon.

The Potential of Alaska Forests

Currently, wood for bassoons manufactured in North America is harvested from the Eastern Deciduous Forest, and the forest is under attack from disease and insects. American elm (*Ulmus americana*) populations were decimated by Dutch elm disease during the 20th century (Karnosky 1979). American chestnut (*Castanea dentata*) populations were destroyed by blight at the same time (Anagnostakis 1987). More recently, ash (*Fraxinus* spp.) has been attacked by the emerald ash borer. Maple (*Acer* spp.) and other common hardwoods have been damaged by the Asian hornedbeetle (Gandhi and Herms 2010), decreasing the supply of high quality wood for bassoons. Also, bassoon manufacturers in North America have used maple (*Acer* spp.) exclusively for the last century to create German system bassoons (Langwill 1971b), which is dangerous because the Eastern Deciduous Forest composition is being altered because of climate change (Dukes, *et al.* 2009), and sugar maple forests in the northeast are steadily declining from environmental factors and disease (Horsley, *et al.* 2002).

Alaska has a forest system comparable in size to the Eastern Deciduous Forest. It has 10 million acres of angiosperm (hardwood) species and 63 million acres of gymnosperms (softwoods) (Smith, *et al.* 2001). Viereck and Little (2007) reported 12 tree species that can attain a girth of at least 18 inches (0.6 m), the minimum girth of maple harvested for resonance wood. Six of the species are hardwoods. The softwoods are not traditionally used for woodwinds, limiting the

choices to six species (Table 1). None of the Salicaceae are suitable for instrument making, because they tend to form voids in the trunk (Pataky 1999) and the wood dries twisted (United States Forest Products Laboratory 1974), which leaves two potential candidates – *Alnus rubra* and *Betula neolaskana.*

In summary, the bassoon's sound is influenced by the instrument's design, the double reed, and the material used to construct the body. The bassoon's timbre is affected by the design and the double reed. Material used to construct the body has a more subtle effect, altering projection and the 'color' of the sound instead of the timbre, because the body of the bassoon does not act as a resonantor and has indirect contact with the vibrating column of air in the bore. However, even indirect contact can have an effect on sound through dampening, which makes material in a bassoon an important component in sound production. Most importantly, the characters currently used to classify resonance woods do not consider this effect, making wood choice a hit-or-miss prospect at best.

From this study, I hope to find more accurate anatomical and mechanical characters to add to the list of known resonant wood characteristics. My questions are three-fold: 1) can characters derived from wood anatomy and mechanical characters derived from tapping (striking an object lightly) be used to better describe bassoon resonant woods than the characters in use now, 2) can the Alaska hardwoods be used as an alternative material for the bassoon, and 3) can lists of potential species and characters to describe bassoon resonant wood be compiled. Answering these questions will potentially reduce the time and money used to create bassoons, along with timber waste (and the need to cut down a resource that is being diminished by disease and whose habitat is being reduced because of human population growth).

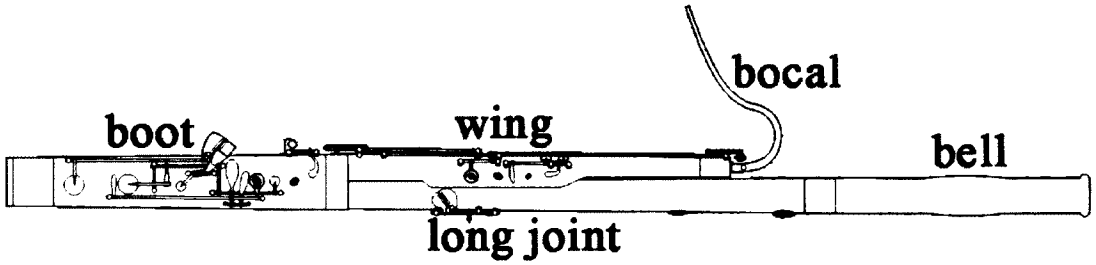

Figure 1 Diagram of a German system bassoon.

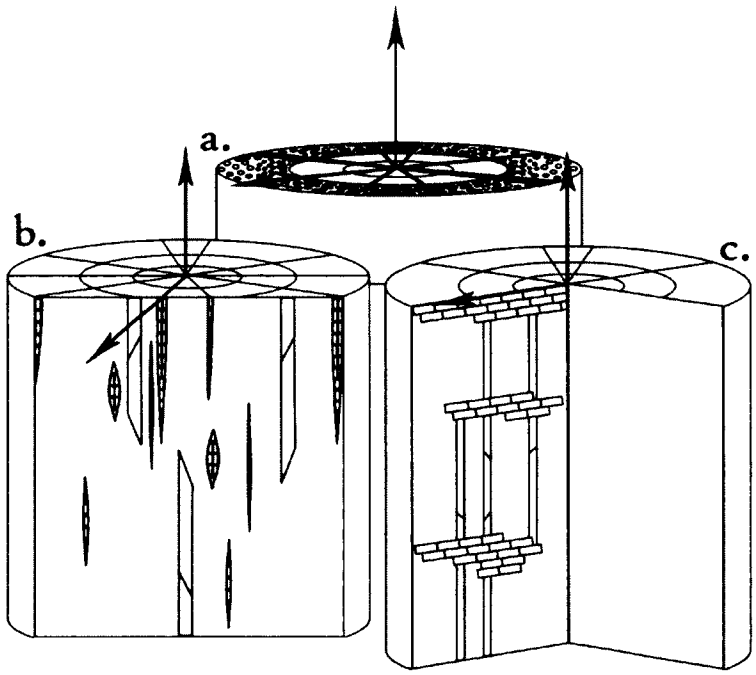

Figure 2 Faces of wood with axes of tree growth indicated by arrows. a. transverse, b. tangential, c. radial.

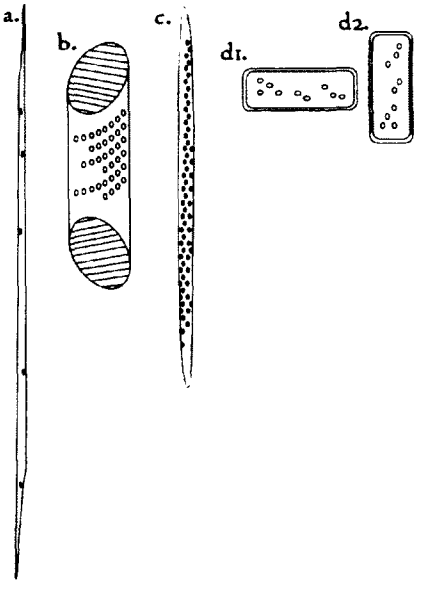

Figure 3 Secondary xylem elements. a) fiber, b. vessel, c. tracheid, d1. ray parenchyma, d2. axial parenchyma.

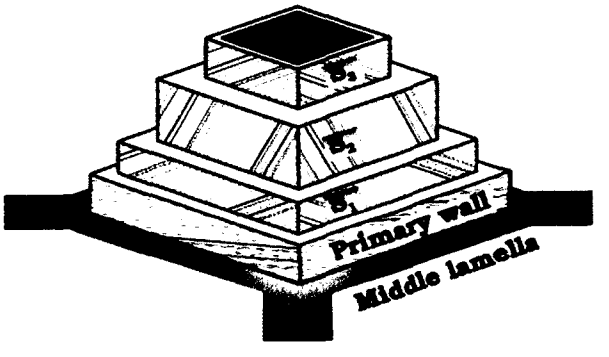

Figure 4 Cell wall layers of secondary xylem with microfibril angles illustrated.

Table 1 List of angiosperm tree species growing in Alaska.

Family	Species	Common name
Betulaceae	*Alnus rubra*	red alder
Betulaceae	*Betula neoalaskana*	Alaska white birch
Salicaceae	*Populus balsamifera*	balsam poplar
Salicaceae	*Populus tremuloides*	quaking aspen
Salicaceae	*Populus trichocarpa*	black cottonwood
Salicaceae	*Salix alaxensis*	felt-leaf willow

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CHAPTER 2: METHODS FOR POLYMER QUANTIFICATION

Introduction

The goal of this project was to find more descriptive characters to depict bassoon resonance wood. Resonance wood is typically described using characters found through hundreds of years of manufacture – straight, clear wood that does not twist or have curving growth rings (Heckel and Heckel 1931). During the 20th century, the mechanical characteristics of elasticity and density began to be used following research done on the violin (Bucur 2006). The two character groups worked well generally; however, some unsuitable woods fall through the vetting process. Fox Products tried black walnut (*Juglans nigra*) in place of the established wood, maple (*Acer* spp.). Black walnut is similar to maple in regards to the traditional and mechanical characters (United States Forest Products Laboratory 1974). It should have worked, but the black walnut bassoon did not project when played in the concert hall.

It seems reasonable to assume that the differences are anatomical. The problems in comparing black walnut to maple are intrinsic. Black walnut is a dark wood, and the extractives and chemical by-products that create the coloration obscure features. Staining has little effect, because the natural coloration masks the stain. Bleaching could help, but there is a possibility that the bleaching process will damage the sample. The extractives and by-products tend to be aromatic in nature, which means they are autofluorescent (Chaffney 2002) and could potentially confound fluorescence microscopy. Maple is a blond wood, meaning there are no extractives or by-products that color wood. The main autofluorescent compounds in maple are pectin and the lignins.

To separate black walnut from maple, accurate and consistent methods of measuring anatomical variables, such as cell characteristics (vessel, fiber, and parenchyma dimensions) and polymer concentration (cellulose, lignin, and hemicellulose) of the cell walls, were needed. Characteristics such as length and width of a cell type (e.g. vessels, fibers, parenchyma) are straightforward to quantify. Polymer concentration is more difficult to quantify, because some polymers stain more easily than others. It is important to consider, because the polymer concentration within a cell wall determines its mechanical properties. It also affects how wood as a whole reacts to a force such as vibration, making polymer concentration a necessary difficulty

to deal with when studying resonance woods. Cellulose can be amorphous – formless – or crystalline in structure, whereas hemicellulose and lignin are amorphous substances (Chaffney 2002). Crystalline structures can be highlighted with polarized light to the exclusion of the amorphous polymers (Murphy 2001), but the amorphous polymers are difficult to separate from each other.

Cellulose can be stained with any number of botanical stains, such as safranin and toluidine blue. Lignin and hemicellulose are more difficult to stain. Lignin stains come in two types – temporary and permanent. The temporary stains are the easiest to use, but they have a very limited life expectancy. Preparing small batches of slides at a time can be time consuming, if large numbers of samples are being studied. Some of the temporary stains also damage the specimen, because acid is used in the process. Phloroglucinol/HCl is the most common stain, but it lasts only 30 minutes (Johansen 1940). Pairing a traditional monochromatic stain, such as safranin, with a calcium chloride mountant also is effective (Herr 1992). Unfortunately, the cover slip cannot be sealed permanently to the mounted specimen, and the calcium chloride crystallizes around the sections, making the section unusable. Permanent stains are the obvious alternative, if you have to keep your slides for a prolonged length of time. There are problems with these stains also, mainly the time needed and the added complication of examining dark wood. Counterstains are the most common permanent stains for lignin, and these can take several hours for a single specimen (Johansen 1940, Ruzin 1999). One of the most common and easiest counterstains is safranin/astra blue (Vazquez-Cooz and Meyer 2002); however, when this study was conducted in 2008, astra blue was not sold in North America through American companies like Fisher Scientific or through Sigma Aldrich, one of astra blue's manufacturers. Another alternative is safranin/fast green (Johansen 1940); however, the recipe for this counterstain requires a mordant – a chemical used to help a stain bind to another substance – to bind fast green to cellulose, and the staining process is time consuming.

Because of the location for this project, finding sources of obtaining samples besides travelling to the lower 48 and collecting wood was necessary. Herbarium specimens are ideal for this purpose, because they represent a species throughout its range. Sampling wood across a species' range is time consuming and expensive, so herbarium slides are often used. Unfortunately, many slides are stained with safranin, which stains crystalline cellulose as well as the amorphous polymers within a cell wall (Johansen 1940). Separating the polymers cannot be done easily with a

traditional light microscope. With the advent of new technology, other methods may work as well or better to illuminate lignin, particularly the confocal microscope.

The confocal microscope (CLSM) uses lasers to illuminate fluorescent chemicals within an organism. In wood, many of the polymers and aromatics are autofluorescent – fluoresce without the help of stains (Chaffney 2002). Lignin is particularly autofluorescent, because it belongs to the aromatic compound group, which is highly fluorescent (Chaffney 2002). Many studies have been done on the autofluorescence of lignin (Albinsson, *et al.* 1999, De Micco and Aronne 2007, Fricker and White 1992, Kitin, *et al.* 2003, Knebel and Schnepf 1991), but there was little consistency in the confocal settings used. The inconsistency is based on the different laser and filter characteristics of each brand and type of CLSM, making the combination of laser and filter needed to extract the lignin signal unique to every instrument. Some microscopes had Raman spectroscopy capability, others had ultraviolet lasers, and some had configurations specific to botanical stains like safranin. The CLSM available for use with this study was bought for medical research and does not have Raman spectroscopy, an ultraviolet laser, or any botanical stain configurations.

The purpose of this study was three-fold: 1) to establish reproducible protocols for extracting polymer deposition data (i.e. location and amounts of lignin, pectin, and the other aromatic compounds in a plant cell wall) from an image; 2) determine which methods work best for deposition imaging; 3) determine if herbarium specimens can be used for deposition imaging. To accomplish these goals, four standard staining protocols were examined on one representative sample of blond wood and four bleaching/staining protocols were examined on one representative sample of dark wood. The effects of stains on CLSM imagery were examined using one representative sample of blond wood stained like an herbarium specimen and explored how the illuminated portions of a dark field image could be isolated with Image J, a free measurement program from the National Institute of Health (Rasband 1997-2011). Three protocols resulted from this study, as well as a configuration of the CLSM for illuminating lignin and the aromatics.

Methods

To determine which light microscopy methods best highlighted polymer deposition, four staining protocols were compared using one representative specimen of unbleached maple, four bleaching protocols were compared using one representative specimen of dark wood, and staining protocols

were compared between bleached and unbleached wood using one representative specimen (Appendix 1). Each specimen came from the trunk wood of the species. The staining protocols are specific to lignin deposition, because lignin staining is the most difficult result to achieve. To determine which confocal configuration works best to highlight lignin on the CLSM available for this project, all the combinations of laser and filters were used to image maple. The light and confocal microscopy protocols were then compared to determine what was the best method to use with the equipment and time available. Using the previously found confocal protocols, an herbarium specimen created from maple was compared to an unstained version of the specimen to determine if the safranin used in the herbarium specimens obscured any autofluorescence. The useable images created with these protocols were measured using the measurement program Image J (Rasband 1997-2011) in two ways, turning the image binary and using the original color image.

Staining, bleaching, and confocal protocols

Staining unbleached specimens. *Acer saccharinum* (silver maple) was chosen to test the efficacy of lignin staining, because it is a clear wood without many extractives. One specimen was sectioned on all faces to 25 μm with an American Optical (AO) 860 sliding microtome. One unstained set was mounted with permount. The other sets were stained following protocols recorded or developed by Ruzin (1999), Johansen (1940), and Yoshikawa *et al.* (2000).

Protocol 1 – Basic Fuschin (0.02 g Basic Fuschin, 2 mL ethanol, diluted to 100 mL with distilled water). This stain is typically used on sections to highlight lignin in specimens with little lignin (i.e. bleached specimens that have had their lignin leached out of the cell wall or those specimens that do not have much lignin naturally). Sections were stained 12 hours at 60° C, washed 12 hours in distilled water, then dehydrated with 95% ethanol. Sections were transferred to 3:1 ethanol/hydrochloric acid for 15 minutes, then washed 24 hours in 100% ethanol (Ruzin 1999).

Protocol 2 – Crystal violet and erythrosin. Crystal violet is supposed to stain lignin, and erythrosin is supposed to stain cellulose. Sections were hydrated in 100% distilled water, stained in 1% aqueous crystal violet for 15 minutes, rinsed in water, destained in picric acid saturated ethanol, rinsed again, and then dehydrated in ethanol. Sections were immersed in saturated solution of erythrosin B in clove oil for 5 minutes, and immersed in a 1:1 ethanol/citrisolv solution for a minute (Johansen 1940).

Protocol 3 – Phloroglucinol. This stain is the most commonly used to highlight lignin. A few drops of a 1% phloroglucinol/ethanol solution were put on sections already mounted to glass slide, and 1 drop of 35% HCl was added (Yoshizawa, *et al.* 2000).

Protocol 4 – Safranin and picro-anilin blue. Safranin, when used in this counterstain, is supposed to highlight lignin, and picro-anilin blue should stain cellulose. Sections were stained in safranin 2 hours, washed with distilled water, and destained with 95% ethanol saturated with picric acid. Sections were then stained for 2 hours in a picro-anilin solution (saturated solutions of picric acid and anilin blue in 95% ethanol were mixed 78% picric acid solution to 22% anilin blue), and washed in alcohol (Johansen 1940).

Bleaching. *Juglans nigra* (black walnut) was chosen to test the efficacy of bleaching, because it is a dark wood and contains many extractives. One specimen was sectioned on all faces to 25 µm with an AO 860 microtome. One unaltered set was mounted with permount. The other sets were bleached with the following methods.

Method 1 – Stockwell's bleach (1 g chromic acid, 1 g potassium dichromate, 10 mL glacial acetic acid, 90 mL distilled water). Sections were immersed in Stockwell's bleach until no coloration was seen (Ruzin 1999).

Method 2 – potassium permanganate/oxalic acid. Sections were immersed in a 1% aqueous solution of potassium permanganate for 10 minutes and then rinsed. Sections were then placed in a 1% aqueous solution of oxalic acid until the potassium permanganate's purple hue disappeared from the sections. The process was repeated until no coloring remained (Ruzin 1999).

Method 3 – commercial bleach. Sections were immersed in a 10% commercial bleach solution for 30 minutes. Sections were then washed in 30% ethanol (Ruzin 1999).

Method 4 – chromic acid. Sections were incubated at 50° C in a 1% aqueous solution of chromic acid for five hours and then washed in water (Ruzin 1999).

Staining bleached specimens. To test how well a bleached specimen stains, one specimen of black walnut was sectioned and four sets of the resultant sections were bleached with either Method 2 or Method 3. These bleaching methods were chosen because of the short time needed

to make them work. Each set was stained with the four previously described lignin staining protocols.

Imaging samples with the CLSM. Each section was photographed using a Zeiss LSM 510 at 40X. An unstained set of sections was also photographed as a reference. All imaging completed on the Zeiss LSM 510 confocal scanning microscope was made using a 488 nm Argon/Neon laser along with a long pass (LP) 530 nm filter (Donaldson, *et al.* 1999). Initially the Find function was used – it determines the settings when fluorescence is first detected at a certain focus. The focus was then changed manually to obtain the brightest image, and Find was selected again. Each setting was recorded after this final step – detector gain, amplifier offset, and amplifier gain. The detector gain controls the brightness and has a range of 78 – 1250; amplifier offset controls contrast and has a range of -2 – 0.1; and the amplifier gain controls the contrast and has a range of 1 – 3 (Matusmoto 2002). The higher the settings, the harder the confocal had to work to locate a signal and the more noise (fluorescence signals coming from the microscope instead of the image) could be in the image. For the purpose of this part of the study, the detector gain was on the target of investigation, since brightness could potentially be correlated to how much lignin is in a sample.

The brightness of the middle lamella – the connecting substance between adjacent cell walls – in the transverse section was used as a gauge for each protocol's and method's effectiveness, since it contains a large amount a lignin (Blanchette 1991). Ease with which the confocal found the signal was also considered in determining which protocol and method worked best.

Using herbarium specimens (the effects of safranin on a CLSM image)

One representative sample of *Acer saccharum* (sugar maple) was sectioned to 25 μm with an AO 860 sliding microtome to simulate an herbarium specimen. This was done instead of using an actual herbarium specimen, because an identical unstained slide could be made, which eliminated any variability introduced from comparing wood from two different trees and potentially harvested at different times of the season. The potential effects of habitat variability could also be eliminated. One set of sections was stained with a 0.1% safranin solution and mounted with Permount. A second set of unstained sections was also mounted with Permount.

Using a Zeiss LSM 510 confocal scanning microscope, the three sets of sections mounted with permount were photographed using all lasers and filters available on the machine. Each section was focused manually with the light microscopy setting. All the lasers were set to 10%, and the first filter was selected (LP 475 on Channel 1). The Find function was used to locate a signal. Each section was refocused to maximize the fluorescence signal using the Continuous scan function. Find was used again to get the final detector gain (DG) settings for the section. The detector gain controls the brightness and has a range of 78 – 1250; a micrograph with a setting higher than 625 was obtained with a weak signal, and one with a DG setting higher than 833 ran the risk being contaminated with noise. Without altering the focus, a new filter was chosen and the Find function used again. These steps were repeated until every filter was used for each laser.

Extracting data from images

Protocol 1 – binary. Images were converted to an 8-bit grey image. The image was then made binary, turning the cell walls black (coded 0) and the empty spaces white (coded 255). A histogram was created and the resulting statistics recorded (i.e. average number of pixels for 0 and 255 and total number of pixels in the image). These numbers were then visually compared to the original image to determine what information was lost in the binary process.

Protocol 2 – color. The image was broken up into three channels – red, blue, and green. A color histogram was created and the resulting statistics recorded (i.e. average number of pixels for 0 and 255 and total number of pixels in the image). The broken image was compared to the original to determine in which channel the data were located.

The two protocols were compared to see which held the most information.

Results and Discussion

The three goals of this study for the doctoral project were to determine which staining, bleaching, and confocal imaging protocols work best for deposition imaging, to determine if herbarium specimens can be used for deposition imaging, and to establish reproducible protocols for extracting polymer deposition data from an image. The staining and bleaching methods needed to be tested on blond and dark woods, because the remaining chapters will involve both wood types; the confocal method from this study had to be developed because the confocal that will be

used in the remaining chapters was bought for medical research and has no botanical settings. Herbarium specimens are typically stained with safranin, which is an autofluorescent botanical dye, and safranin could overwhelm the autofluorescent signal from the polymers used to build the cell wall, particularly lignin. A reliable way to measure all the images that will be produced from these protocols is needed for the remaining chapters. Three protocols were developed as a result of this study.

Staining, bleaching, and confocal protocols

Stained unbleached specimens. Basic fuschin is supposed to stain lignin preferentially. In the bright field image (Figure 1a.), this stain seemed to cover every surface indiscriminately. Using a polarizing filter, the dark field image (Figure 1b.) showed that basic fuschin did stain the middle lamella. The confocal image (Figure 2b.) shows the brightest region to be the cell wall. Since the middle lamella has the most lignin and shines brightest unstained, basic fuschin does not boost lignin fluorescence. Comparing the automatic settings (Table 1), the detector gain did decrease when the stain was added. This could be explained by the larger area the cell wall occupies compared to the middle lamella.

Crystal violet is supposed to stain lignin preferentially, and erythrosin is supposed to prefer cellulose. Looking at the bright field image (Figure 1c.), crystal violet seemed to cover most areas evenly, whereas the erythrosin stained isolated patches on the section. Using a polarizer, the dark field image (Figure 1d.) the crystal violet did stain the middle lamella. In the confocal image (Figure 2c.), the areas covered in crystal violet were bright spots, particularly the cell wall. The areas covered with erythrosin were dark patches without any fluorescence. Since the middle lamella and its lignin were the targets, neither crystal violet nor erythrosin helped boost the fluorescence of lignin. Comparing this staining protocol to the unstained specimen (Table 1), the detector setting was lower for the stained specimen.

Phloroglucinol is supposed to stain only lignin. Looking at the bright field image (Figure 1e.), the stain appeared to cover every surface with an even hue. Using the polarizer, the dark field image (Figure 1f.) showed that the stain did indeed stain the middle lamella. In the confocal image (Figure 2d.), the middle lamella comprised the brightest area. Comparing this image to the unstained version (Figure 2a.), the stain did not overwhelm the middle lamella and, by association, lignin. Looking at the automatic settings (Table 1), the settings for the

phloroglucinol specimens were higher than the unstained specimens. Even though it stained the lignin, it also weakened the signal. This could be caused by the use of hydrochloric acid, or the stain may not be fluorescent at all.

Safranin, when used as a counterstain, is supposed to stain lignin; picro-anilin blue is supposed to stain cellulose. Looking at the bright field image (Figure 1g.), the two stains appeared to blend together, coating the section evenly. Under dark field conditions (Figure 1h.), safranin did not seem to highlight the middle lamella. In the confocal image (Figure 2e.), the brightest spots seemed to be the areas stained by safranin, which were mostly cell walls. The picro-anilin blue stained cells did not seem to shine through, since the areas of this stain were dark. Neither stain boosted the brightness of the middle lamella, suggesting the stains targeted cellulose more. Comparing the settings (Table 1), the detector settings for the stains were lower than the unstained setting. Like the basic fuschin, this could be explained by the larger cell wall area to that of the middle lamella.

Bleached specimens. The unbleached walnut sections (Figure 3a.) were brightest in the middle lamella. Like the unstained walnut, the automatic settings were in the middle range (Table 2).

The image of Method 1 (Figure 3b.) was fainter than the unbleached image, especially in the middle lamella. The brightest spots turned out to be the cell wall, although most of the wall disappeared compared to the unbleached specimen (Figure 3a.). Method 1 appeared to leach lignin out to a considerable degree and made the sections much less fluorescent. Compared to the unbleached specimen, the detector gain setting recorded for Method 1 was higher (Table 2), meaning that it was more difficult for the microscope to detect a fluorescence signal.

In the image of Method 2 (Figure 3c.), the middle lamella was brighter than the secondary cell wall, a result comparable to the results from the unbleached specimen (Figure 3a.). Compared to the unbleached specimen, the specimen treated with Method 2 had a higher detector gain setting (Table 2), which meant it was more difficult to detect a fluorescence signal. Combining the visual inspection of the image with the detector gain comparison, it seemed Method 2 leached some lignin. When compared to specimens treated with Method 1, the detector gain setting was lower, which would indicate Method 2 leached less lignin than Method 1.

The middle lamella was brighter than the cell wall in the image of the specimen treated with Method 3 (Figure 3d.), a result comparable to the results from the unbleached specimen. The detector gain setting for the specimen treated with Method 3 (Table 2) was higher than both the unbleached specimen and the specimen treated with Method 2, meaning Method 3 leached more lignin than Method 2. Compared to Method 1, the detector gain setting for specimens treated with Method 3 was lower and did not leach as much lignin.

Method 4 macerated (disintegrated) the transverse section before bleaching was complete. Many sections were treated with the same method, and all macerated before bleaching was completed.

Stained bleached specimens. The brightest area of the reference sample – an unstained, unbleached walnut section (Figure 4a.) – was the middle lamella, where much of the lignin is located. The settings were in the middle of the range (Tables 3, 4).

The basic fuschin stained sample bleached with Method 2 (Figure 4b.) was brightest in the cell wall region. Unlike the stained, unbleached sample, the middle lamella did not show. Since viewing lignin is the goal, this method was deemed unsuccessful. The detector gain setting was higher in this section than in the unaltered specimen (Table 3). The basic fuschin stained specimen bleached with Method 3 (Figure 5b.) was also brightest in the cell wall region. This image was comparable to the image of the section altered with Method 2 and differed from the unaltered specimen by not having a bright middle lamella. Looking at the automatic settings (Tables 3, 4), the basic fuschin stained Methods 2 and 3 settings were equivalent to each other and higher than the unaltered section. Bleaching methods seemed to be interchangeable when basic fuschin was used as a stain.

The crystal violet/erythrosin stained sample bleached with Method 2 (Figure 4c) was brightest in the cell wall region. This was contrary to the desired brightness of the middle lamella, seen in the unaltered specimen. The sample bleached with Method 3 (Figure 5c) was also brightest in the cell wall area. Both had lower settings compared to the unaltered specimen, which could be explained by the total area encompassed by the cell walls (Tables 3, 4). The crystal violet/erythrosin stained sample bleached with Method 3 had a higher setting than the sample bleached with Method 2 and stained with the same counterstaining procedure. Compared to the basic fuschin sections, the crystal violet/erythrosin samples had lower settings. It did not seem to matter which bleaching technique was used.

The phloroglucinol stained sample bleached with Method 2 (Figure 4d) was brightest in the middle lamella region, which is where much of the lignin is found. The sample bleached with Method 3 (Figure 5d) was also brightest in the middle lamella. Compared to the unaltered sample, the settings for stains samples bleach with Methods 2 and 3 were higher (Tables 3, 4). The detector gain setting for the sample altered by Method 2 was lower than the setting for the sample altered by Method 3. Both were much higher than the detector gain settings for the other staining protocols. Again the two bleaching schemes seemed to produce equivalent results.

The safranin/picro-anilin blue stained section bleached with Method 2 (Figure 4e) was brightest in the cell wall areas covered with safranin. Unlike the unaltered sample, the middle lamella was not bright. The sample bleached with Method 3 (Figure 5e) was visually similar to the sample bleached with Method 2. Detector gain settings were higher in the sample treated with Method 2 (Table 3) than in the unaltered sample, and detector gain settings were lower in the sample treated with Method 3 (Table 4) than in the unaltered sample. Both safranin/picro-anilin blue stained sections had lower detector gain settings than the other samples treated with the other stains.

Overall, the middle lamellae were the brightest areas in the micrographs of the unstained sections. The secondary walls were the brightest areas in the micrographs of the stained section when the cell walls of the fibers and vessels were the dominant features of the image. In the micrographs that showed only the ray parenchyma, the contents of the parenchyma were the brightest areas for both unstained and stained sections.

None of the staining protocols isolated lignin in the samples. Most of these protocols were designed for paraffin embedded sections cut to a thickness of less than 10 μm, and the sections used in this study were cut thicker and were not embedded. The thickness of the sections may be impeding the exposure of the lignin, but obtaining a thin section would require embedding the wood with paraffin, which is time consuming. The bleaching also worked to an extent, but there was too much leaching of the lignin from the sections. Since lignin is one of the polymers being examined for the remaining chapters, bleaching would compromise the data. The confocal is ideal for deposition imaging (the location and concentration of the aromatic polymers located in the cell wall) in terms of time saved by not staining. The CLSM cannot show the crystalline structures, however. Crystalline structures can be shown using a light microscope without

staining, as long as the microscope has polarizers. The same slide prepared for the confocal can be used with the light microscope and polarizers, saving on preparation time.

Using herbarium specimens (the effects of safranin on a CLSM image)

Confocal microscopes have two types of filters through which light passes, long pass (LP) and band pass (BP). Each type filters specific wavelengths of light, eliminating the other wavelengths from the confocal image. Confocal microscopes also have a variety of lasers for light sources. The variety depends on the brand of microscope and options available for specific units. Since the confocal used in this study did not have a filter/laser preset for plant cells or specimens treated with botanical stains, every combination of laser and filter had to be examined. The detector gain settings provided a clue on the success of each combination. If the combination needed a high setting to produce an image, then too many wavelengths were filtered out and noise (i.e. light from the room or computer screen) may have been used to produce the image.

Long pass (LP) filters – unstained vs. stained. Lignin in the walls could not be separated from the other aromatics using the LP filters, but the aromatics could be separated spatially. The wall contents of the unstained specimens were best separated from the parenchyma lumen contents by the laser/filter combination of 477 nm or 488 nm/LP 475 and LP 505 – LP 585 or 514 nm/LP 505/LP 530 (Figure 6). The wall contents of the unstained specimens were best separated from the parenchyma lumen contents by the laser/filter combination of 477 nm or 488 nm/LP 475 and LP 505 – LP 585 or 514 nm/LP 505/LP 530 (Figure 7).

Band pass (BP) filters – unstained vs. stained. Lignin could not be separated from the other wall aromatics using BP filters, but the aromatics could be separated spatially. The wall contents of the unstained specimens could be best separated from the parenchyma lumen contents by the laser/filter combination of 477 nm/ BP 530-600 to BP 560-615. They can also be separated by 488 nm/BP 475-525/BP 530-600 to BP 585-615 and 514 nm/BP 475-525 to BP 505-530 and BP 505-550 to BP 560-615 (Figure 8). The aromatics from the stained specimens could be separated using the same combinations as the unstained specimens (Figure 9).

Detector gain (DG) settings – unstained vs. stained. The unstained specimens produced a strong signal using all the lasers except 458 nm with the filters LP 475 to LP 530. All the lasers except

633 nm caused the unstained specimens to emit a strong signal with the filters BP 530-600 to BP 505-550 (Table 5). The stained specimens produced similar results (Table 6).

Combining the information from both the images and settings, the best lasers to use were 477 – 514 nm. The filter choices for the parenchyma contents were consistent throughout with LP 475 and BP 475-525. Extracting the cell walls was easiest with LP 505, LP 530, and BP 530-600 filters.

Herbarium specimens can be used for the doctoral study, but the safranin used to stain the sections overwhelmed the settings. Overwhelming the settings means that any data obtained from the image would skew the statistics (i.e. the average concentration of aromatic material in the cell wall and the cell lumen) higher than they should be. The herbarium specimens can still be used to obtain crystalline cellulose information from a light microscope equipped with polarizers.

Extracting data from images

The conversion of the RGB image to binary merged the grey portions with the brightest spots in the wall, whereas splitting the original color image into individual channels separated out the brightest spots of the walls without merging the grey area (Figure 10). The information from the binary image's histogram was interpreted as follows – the mean corresponded to the color level where the average number of pixels were found (0 and 255) and the mode corresponded to the color level with the most pixels (Table 7). The RGB image histogram was interpreted as follows – the red channel mean corresponded to the color level where the average number of pixels were found (0 – 255); the red mode corresponded to the color of brightest luminescence; the green channel mean corresponded to cellulose below the surface of the section; and the blue channel had no usable information (Table 8).

Wall and parenchyma contents could be broken up by separating the channels (Figure 10). Information from the color histogram could be interpreted as follows – the red channel corresponded to the wall contents, the mean corresponded to the color level with the average number of pixels, and the mode corresponded to the color level with the highest concentration of pixels (0 – 255); the green channel was a merged channel between wall and parenchyma; the blue channel corresponded to the parenchyma contents, the mean corresponded to the color level with the average number of pixels, and the mode corresponded to the color level with the highest

concentration (Table 8). Comparing the confocal image to a polarized light image and a traditionally stained image, lignin was most likely in the red channel. By a process of elimination, the blue channel most likely held the byproducts and waste produced by the tree during its life cycle.

Both the binary image and the unaltered color image could be used to measure deposition. However, some data were probably lost converting a color image to binary. The subtle gradations in color from bright to dark could hold important data to the remaining chapters. A color image is more complicated to measure with its different channels, but it also retains those gradations.

In summary, the most information was obtained from unbleached, unstained specimens. Bleaching damaged the specimens or leached the aromatic polymers out of the cell wall. Staining did not differentiate between the crystalline cellulose and amorphous polymers enough to measure. The confocal microscope produced clearer images for measurement of amorphous polymer deposition than the light microscope, and the light microscope imaged crystalline cellulose better using polarized light. With the confocal microscope, the herbarium specimens could not be used to examine the deposition and concentration of aromatic polymers (i.e. lignin, pectin, etc). The color images provided the most information about polymer deposition and concentration. As a result, three protocols were developed for use in the remaining chapters – one for imaging unstained sections with the Zeiss LM510 at UAF, one for imaging crystalline substances in a compound light microscope, and one for measuring images from the two microscopes using Image J.

PROTOCOL 1: Imaging unstained sections on a Zeiss LM510.

1. Set 488 nm laser to 20%.
2. Load the LP 475 and LP 530 filters.
3. Set pinhole to minimum to image at a few microns thickness. A large pinhole will image a thicker section and introduce noise from outside the microscope.
4. Set the detector gain, amplifier gain, and amplifier offset to the middle of their ranges. The settings have to be high enough to get a reading from a blond wood, yet low enough to avoid introducing noise.
5. This project used the 40X ocular. Other oculars were tried, and all worked well.
6. Focus the section manually with the halogen light.
7. Take a single frame of the section.
8. Putting the imager on Continuous frames, focus the microscope for the brightest and clearest image.
9. Stop the Continuous frames.
10. Set the frame average to 4 or 8 and take another single frame. The frame averaging will smooth out the pixels for a plate-worthy image.
11. Save the image as a .tif to avoid lossy compression (an encoding method that compresses data by discarding some of it (Sayood 2012)).

PROTOCOL 2: Quantifying surface crystalline cellulose with Image J.

1. Load the image into Image J and make sure it is in RGB.
2. Under Analyze, select Tools. In Tools, select Color Histogram.
3. A visual and numerical summary of the histogram will be created for all the channels. Selecting List in the Histogram window will open a table with the pixel counts for every channel in the histogram. The red channel is generally the most useful for cellulose deposition. Sometimes the blue channel will be the better alternative, because the section was cut badly or the sample is from a young tree. My interpretation of the data is in the following table.

Variable from Histogram	Description	Interpretation
rMean	the average of all the color levels containing pixels	this is the average color of the image overall
rMode	the color level with the most pixels	this is the color that is most dominant in the image

4. Find the number of pixels in the color level indicated by rMode. Use the following formula to estimate the amount of surface cellulose in a darkfield image:

$$\%\ \text{surface cellulose} = \frac{(\text{total number of pixels in image} - \text{number of pixels in rMode})}{\text{total number of pixels in image}} \times 100$$

PROTOCOL 3: Quantifying aromatic polymer deposition with Image J.

1. Load the image into Image J and make sure it is in RGB.
2. Under Analyze, select Tools. In Tools, select Color Histogram.
3. A visual and numerical summary of the histogram will be created for all the channels. Selecting List in the Histogram window will open a table with the pixel counts for every channel in the histogram. The red and blue channels are the most important for aromatic polymer deposition. My interpretation of the data is in the following table.

Variable from Histogram	Description	Interpretation
rMean	the average of all the color levels containing pixels in the red channel	this is the average color of the wall contents
rMode	the color level with the most pixels in the red channel	this is the most dominant color of the wall contents
count from 0	the number of pixels occupied by black	this can be used to determine the percentage of luminescence of the wall contents by creating a ratio using the total Count
bMean	the average of all the color levels containing pixels in the blue channel	this is the average color of the parenchyma lumen contents
bMode	the color level with the most pixels in the blue channel	this is the most dominant color of the parenchyma lumen contents

4. Find the number of pixels in the color level indicated by rMode or bMode. For tropical specimens, you may have to determine the beginning of the black zone using the histogram image because the thick walls are overwhelming the fluorescence levels. Use the following formula to estimate the amount of surface cellulose in a darkfield image:

% channel luminescence =

$$\frac{(\text{total number of pixels in image-number of pixels from 0 to rMode level or bMode level})}{\text{total number of pixels in image}} \times 100$$

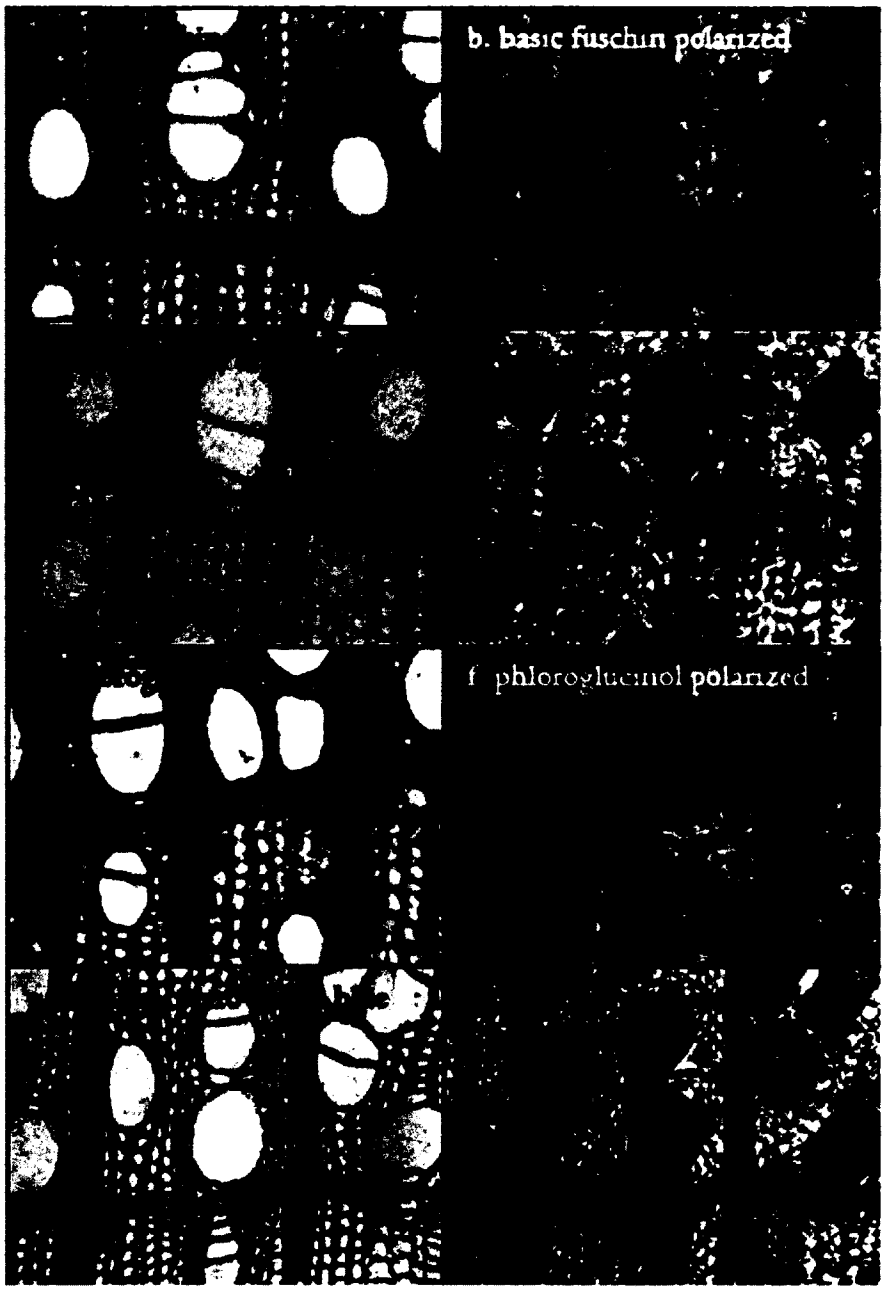

Figure 1 Light micrographs of stained maple sections, direct light (bright field) and indirect light (dark field). Staining protocols used for each image are written in the upper left corner. Bright field images are a, c, e, and g. Dark field images are b, d, f, and h.

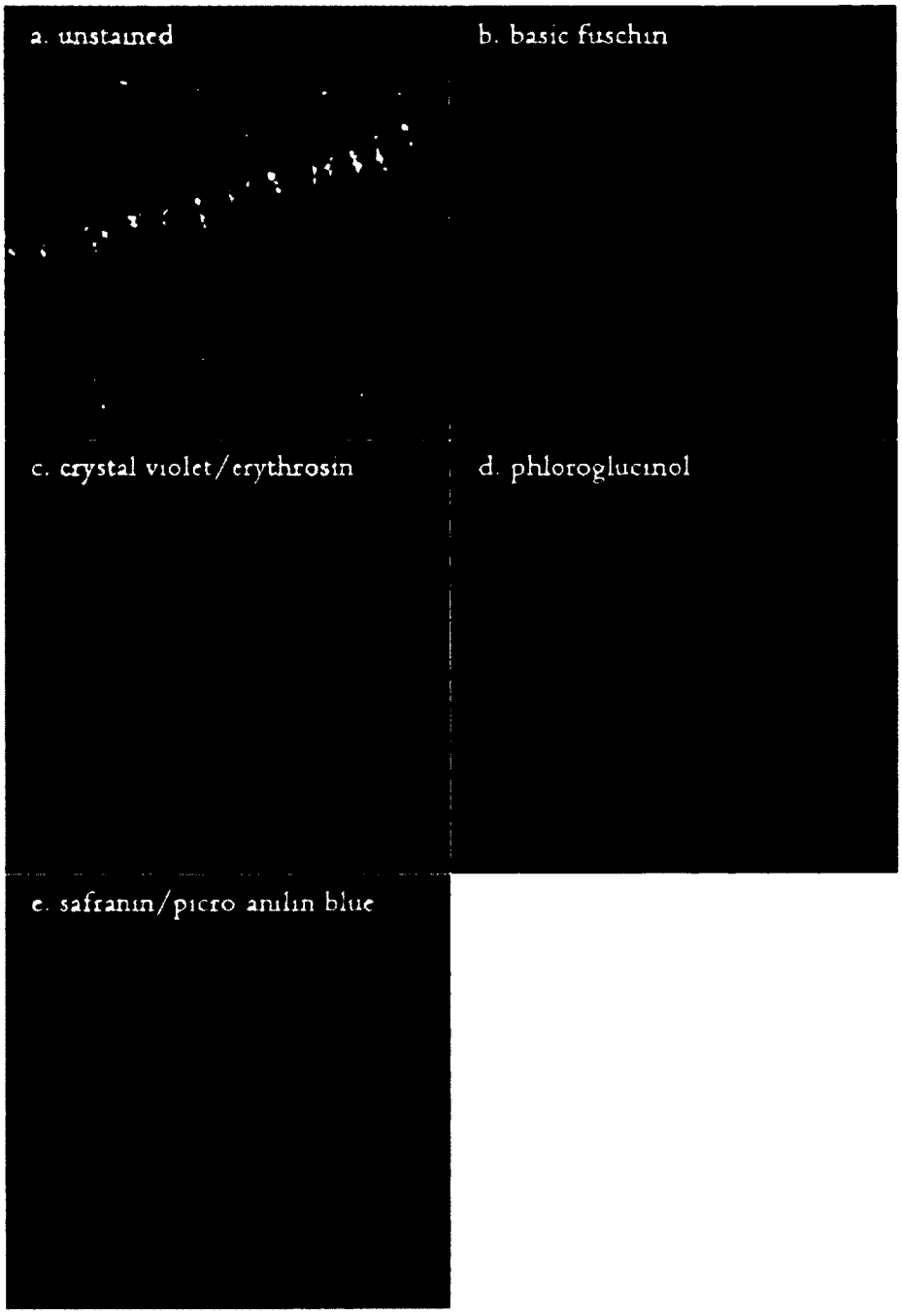

Figure 2 Confocal micrographs of stained silver maple sections. Staining protocols used for each image are indicated in the upper left corner.

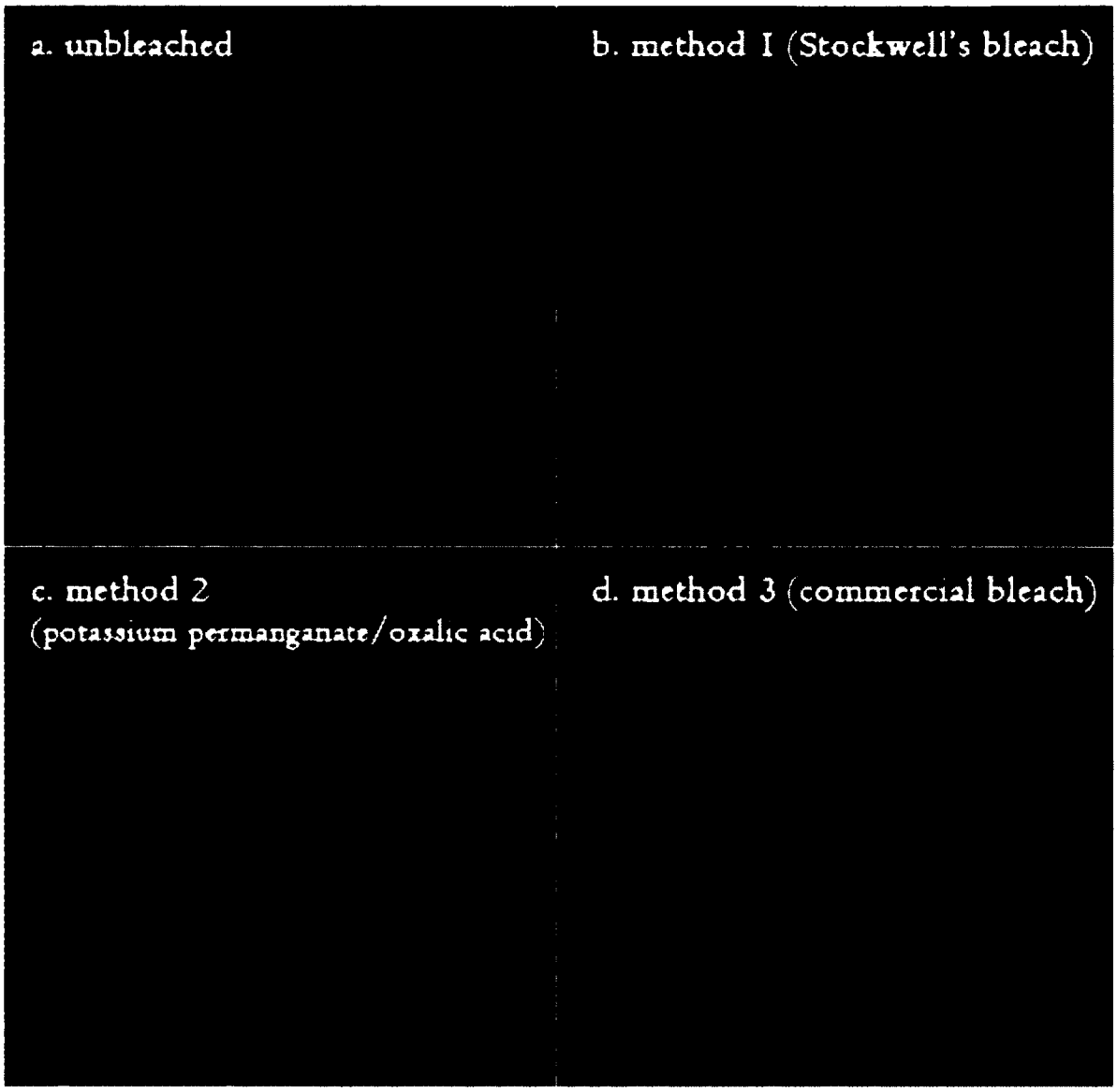

Figure 3 Confocal micrographs of bleached walnut sections. Beaching methods used on each image are indicated in the upper left corner.

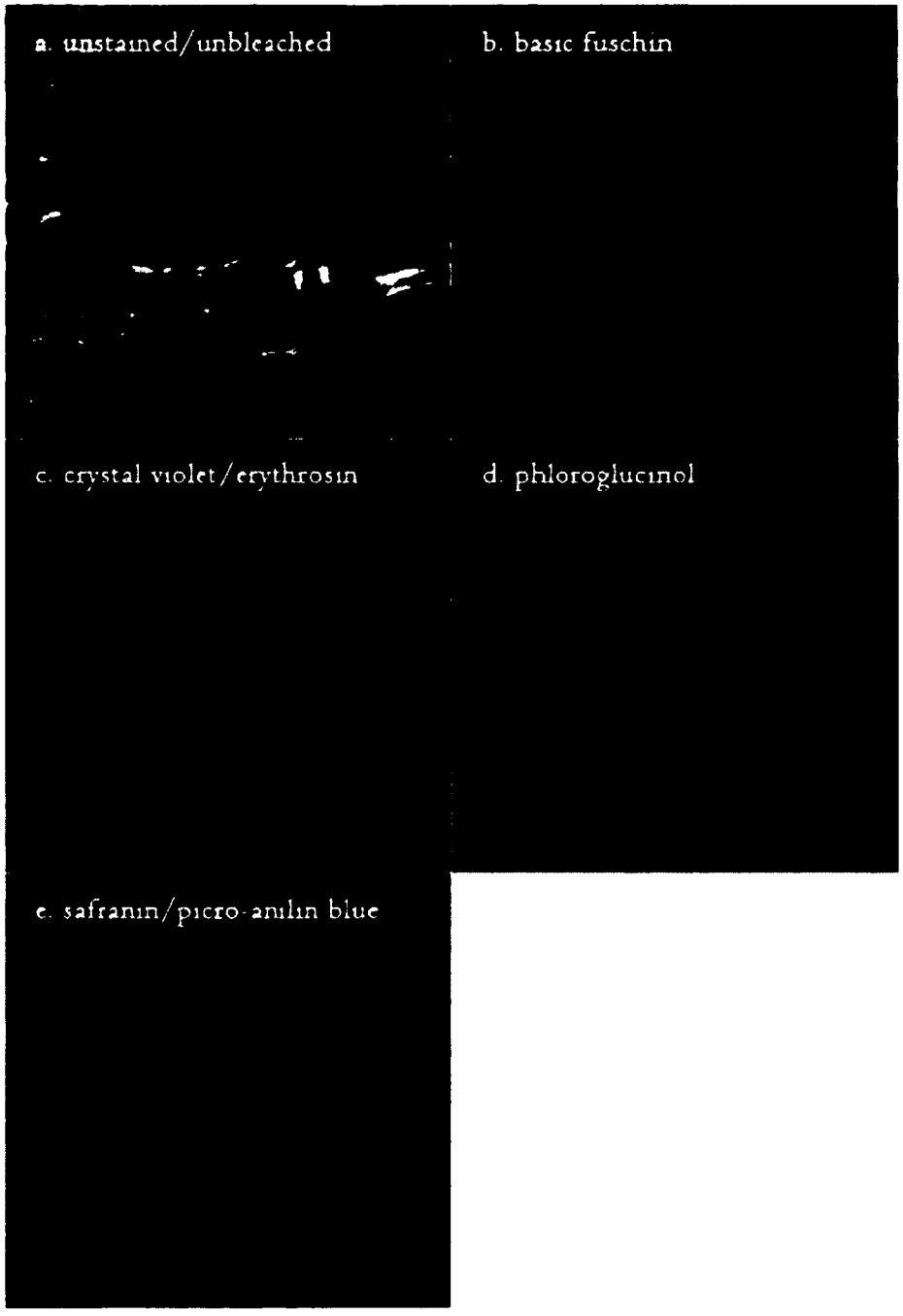

Figure 4 Confocal micrographs of walnut sections bleached with Method 2 and stained with all protocols. Staining protocols used on each image are indicated in the upper left corner.

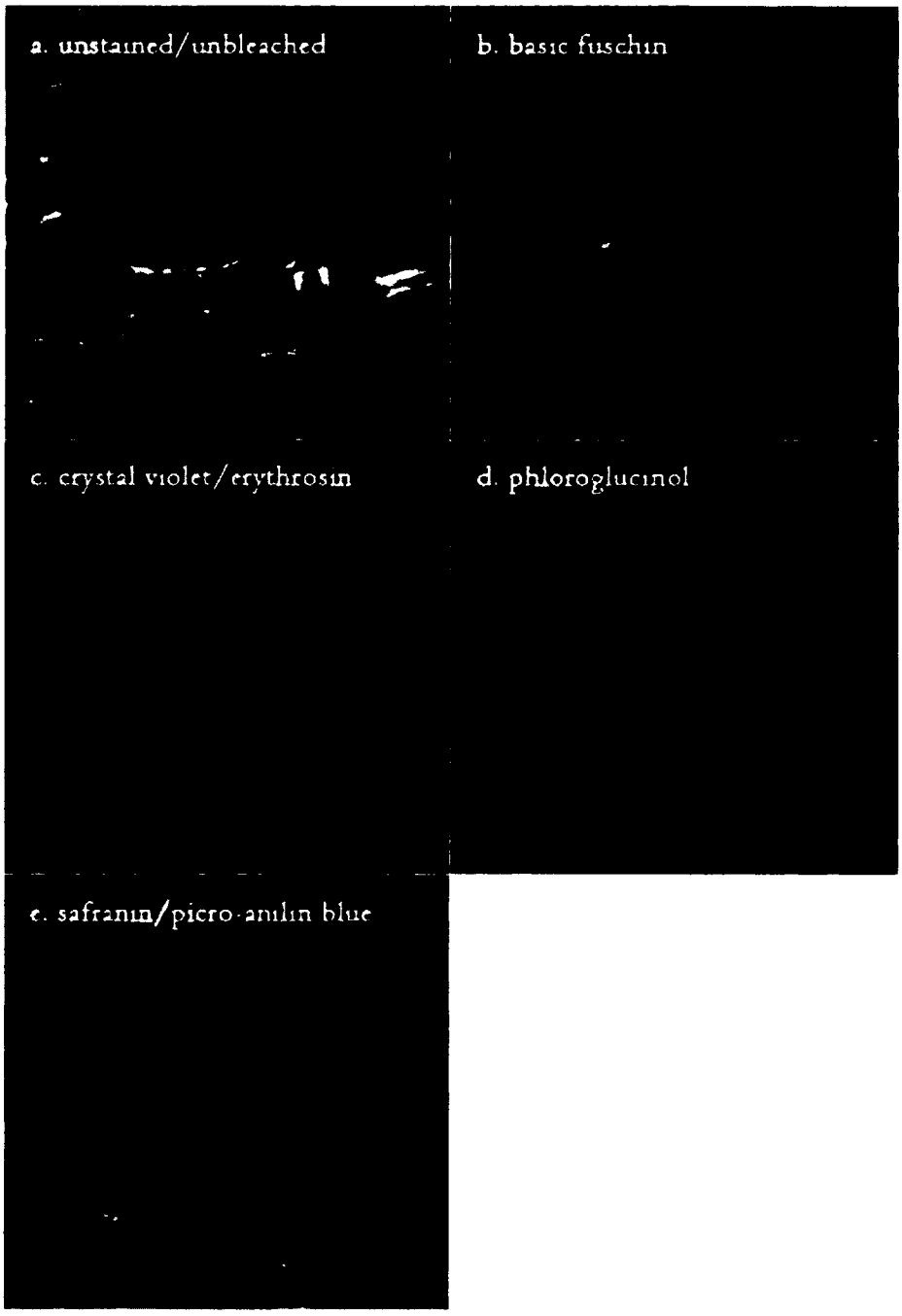

Figure 5 Confocal micrographs of walnut sections bleached with Method 3 and stained with all protocols. Staining protocols used on each image are indicated in the upper left corner.

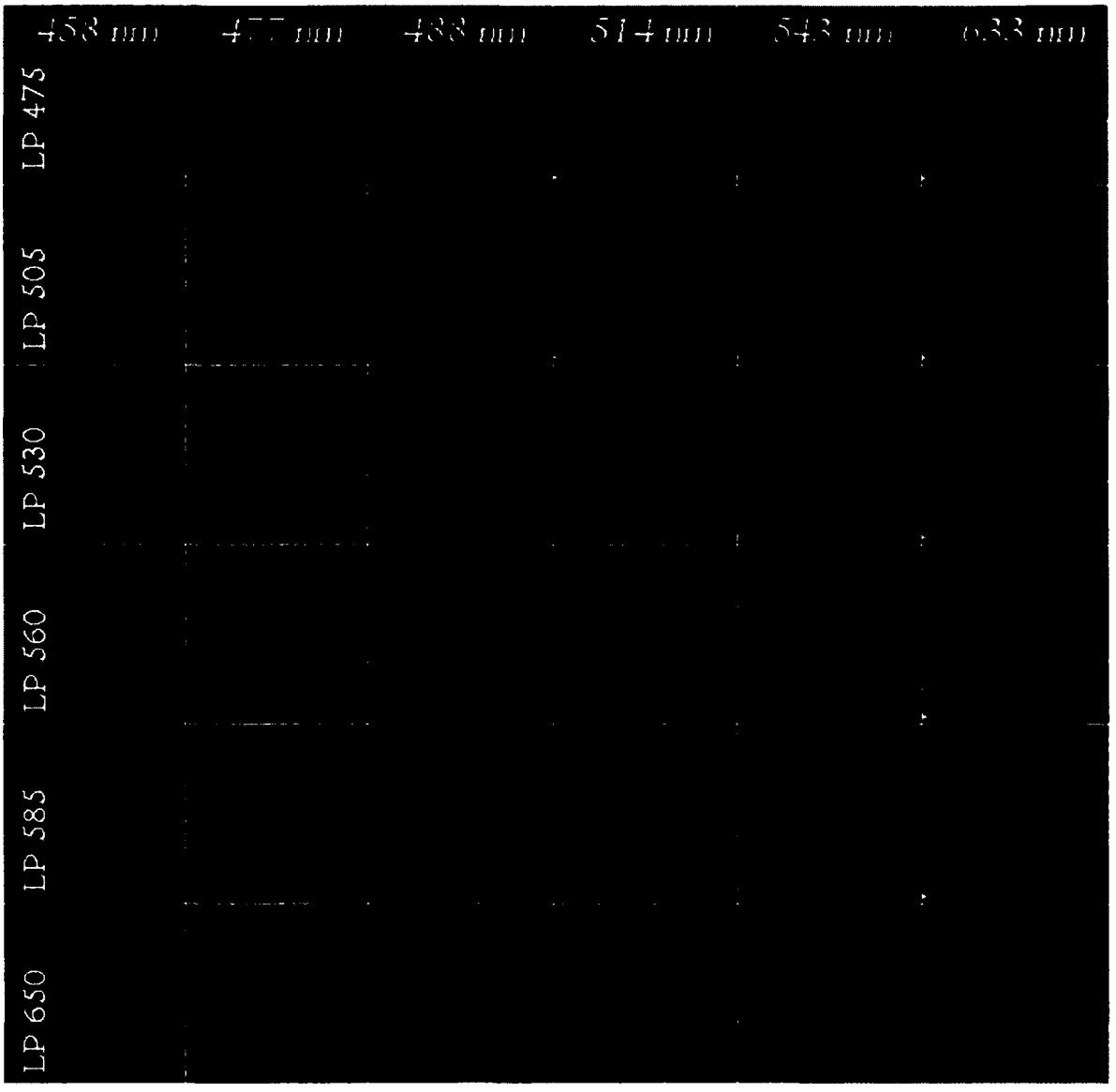

Figure 6 Micrographs of unstained sections created with long pass (LP) filters. Filter type used for each image is indicated at the beginning of the row, and laser used for each image is indicated at the top of the column.

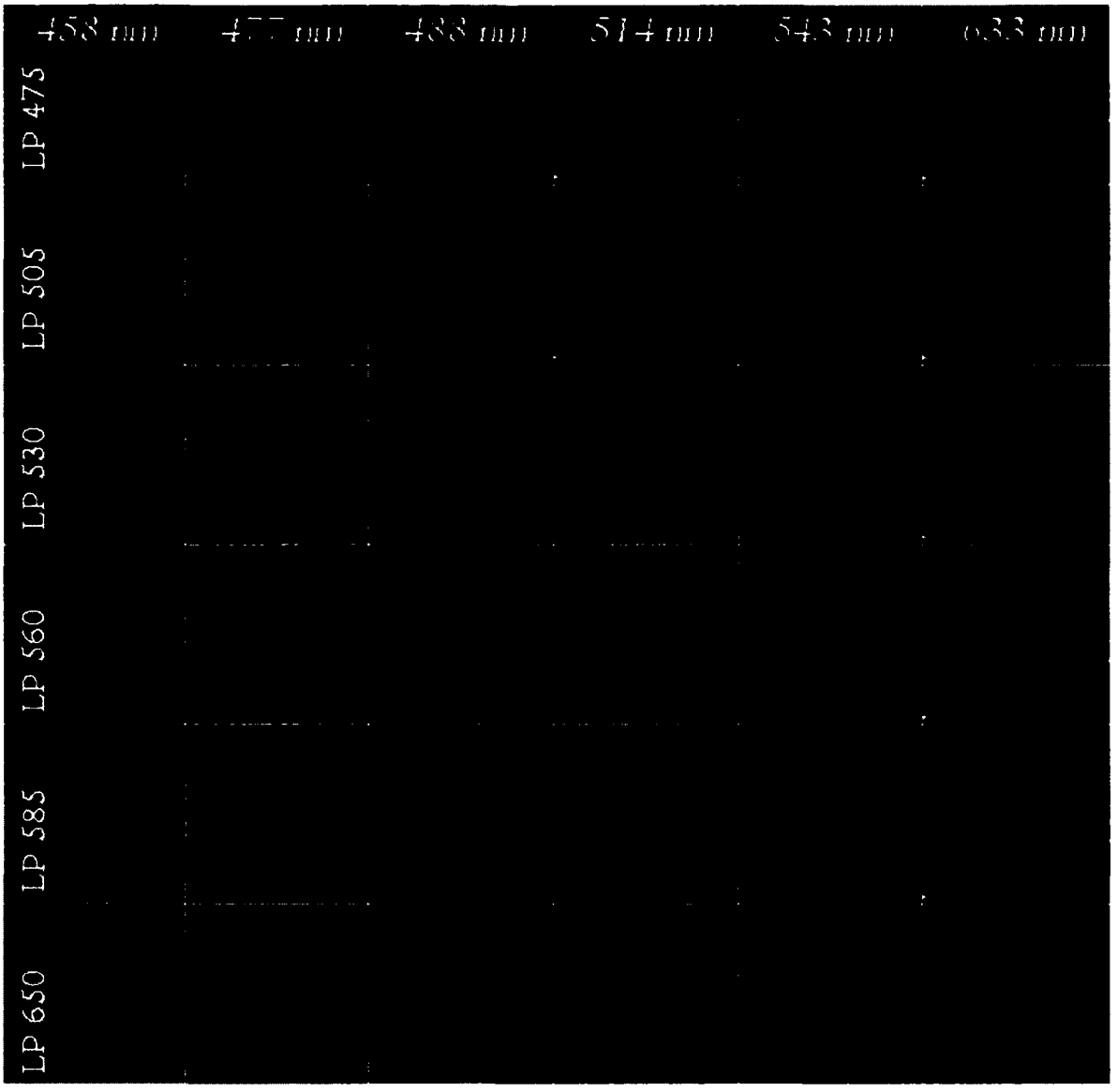

Figure 7 Micrographs of stained sections created with long pass (LP) filters. Filter type used for each image is indicated at the beginning of the row, and laser used for each image is indicated at the top of the column.

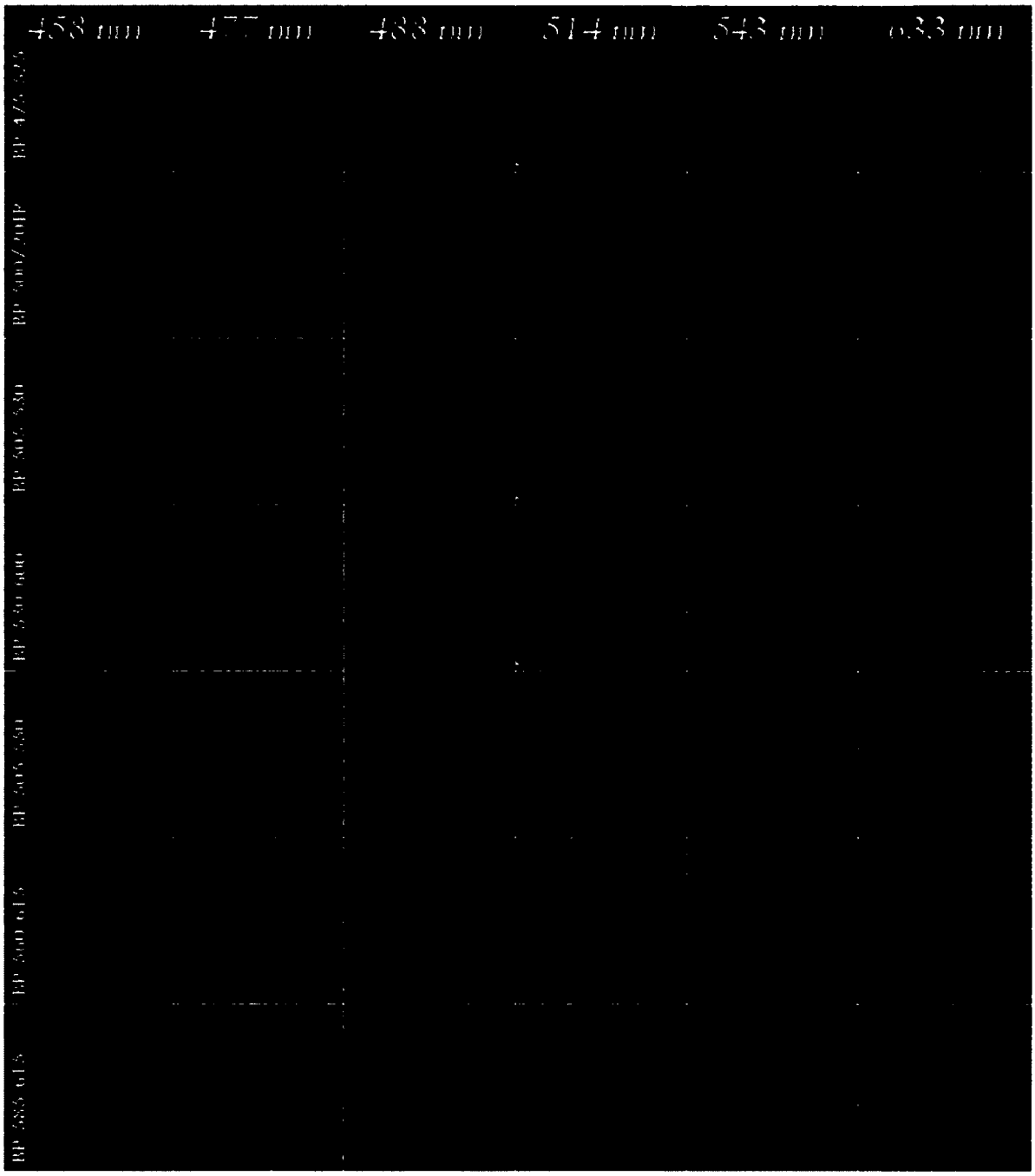

Figure 8 Micrographs of unstained sections created with band pass (BP) filters. Filter type used for each image is indicated at the beginning of the row, and laser used for each image is indicated at the top of the column.

Figure 9 Micrographs of stained sections created with band pass (BP) filters. Filter type used for each image is indicated at the beginning of the row, and laser used for each image is indicated at the top of the column.

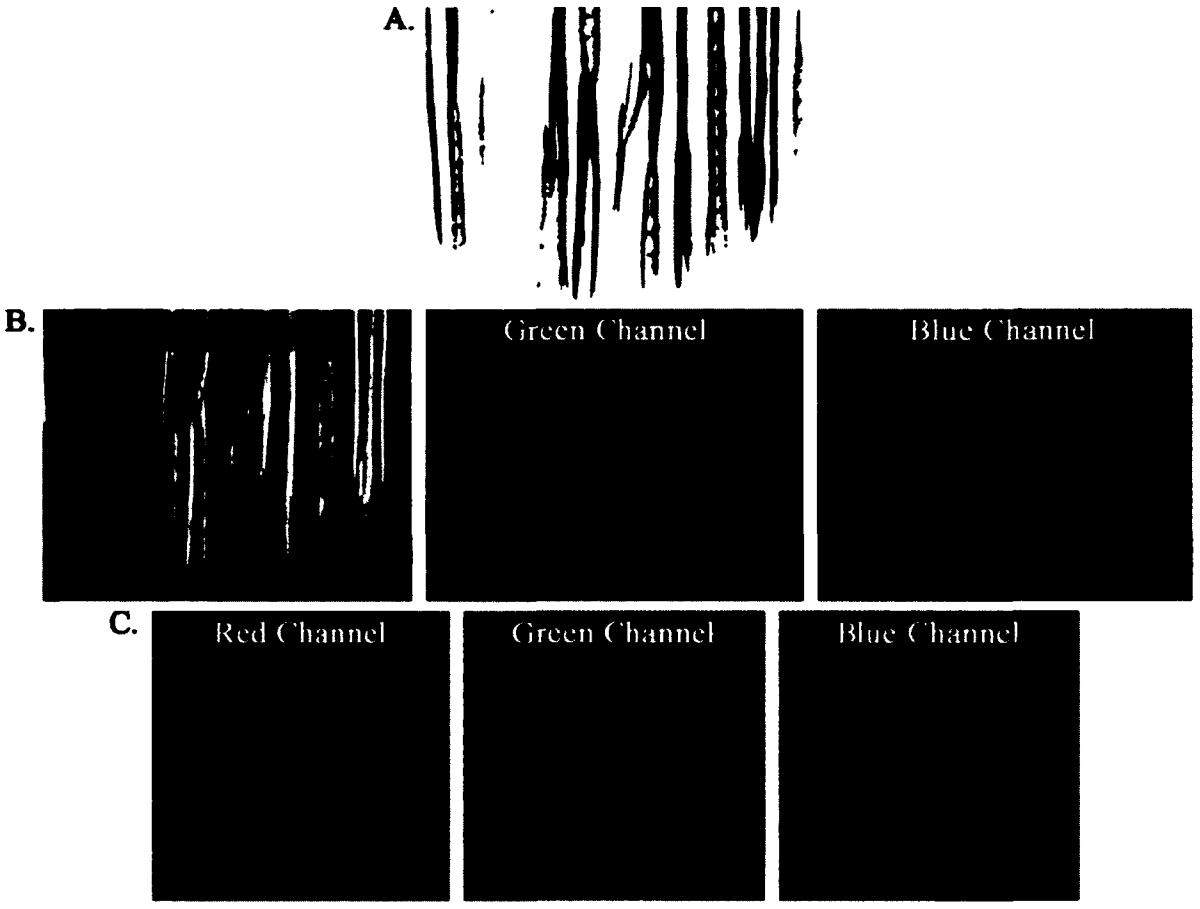

Figure 10 Image of black walnut turned binary (A.) and unaltered color image broken into three channels (B.) for measurement in Image J.

Table 1 Automatic settings obtained with the Find function for stained maple sections.

Stain	Detector Gain	Amplifier Offset	Amplifier Gain
unstained	**688**	**-0.16**	**1.02**
basic fuschin	532	-0.16	1
crystal violet &erythrosin	577	-0.16	1.01
phloroglucinol	741	-0.16	1.01
safranin & picro-anilin blue	423	-0.152	1.03

Table 2 Automatic settings obtained with Find function for bleached walnut sections.

Bleaching method	Detector Gain	Amplifier Offset	Amplifier Gain
unbleached	**733**	**-0.152**	**1**
Method 1	961	-0.152	1.14
Method 2	798	-0.175	1
Method 3	802	-0.152	1
Method 4	na	na	na

Table 3 Automatic settings obtained with the Find function for stained walnut sections bleached with Method 2.

Stain	Detector Gain	Amplifier Offset	Amplifier Gain
unstained, unbleached	**552**	**-0.223**	**1.01**
basic fuschin	610	-0.16	1
crystal violet &erythrosin	566	-0.16	1
phloroglucinol	774	-0.152	1.01
safranin & picro-anilin blue	581	-0.175	1

Table 4 Automatic settings obtained with the Find function for stained walnut sections bleached with Method 3.

Stain	Detector Gain	Amplifier Offset	Amplifier Gain
unstained, unbleached	**552**	**-0.223**	**1.01**
basic fuschin	611	-0.168	1
crystal violet &erythrosin	584	-0.16	1.02
phloroglucinol	798	0.152	1.08
safranin & picro-anilin blue	506	-0.168	1.05

Table 5 Detector gain (DG) settings for unstained section. ǂDetector gain (DG) settings for unstaiǟ > 833, * prominent ray parenchyma.

Filter	458	477	488	514	543	633
LP475	688	548*	411*	455*	577*	467*
LP505	1016ǟǂ	753	632	450*	580*	467*
LP530	989ǟǂ	771	643	736	573*	463*
LP560	968ǟǂ	803	669	757	969ǟǂ	475*
LP585	748	843ǟǂ	694	792	996ǟǂ	471*
LP650	1046ǟ	994ǟǂ	837ǟǂ	930ǟǂ	1115ǟ	1080ǟ
BP475-525	811	479*	391*	423*	1001ǟ	1018ǟ
BP500/20IR	902ǟ	896ǟ	725	652*	1076ǟ	1065ǟ
BP505-530	872ǟ	862ǟ	769	427*	1076ǟ	1087ǟ
BP530-600	775	741	632	727	549*	891ǟ
BP505-550	798	771	672	433*	541*	905ǟ
BP560-615	907ǟǂ	853ǟǂ	735	827	1019ǟǂ	1043ǟ
BP585-615	979ǟǂ	929ǟǂ	811	904ǟǂ	1043ǟǂ	1071ǟ

Table 6 Detector gain (DG) settings for stained section.ǂ signal weaker in unstained sections, ǟ > 833, * prominent ray parenchyma.

Filter	458	477	488	514	543	633
LP475	676	581	434	474	566*	395*
LP505	680ǂ	601	489	474	577*	400*
LP530	682ǂ	601	493	524	568*	391*
LP560	691ǂ	611	498	534	669ǂ	399*
LP585	710	637ǂ	523	558	701ǂ	399*
LP650	836ǟ	757ǂ	631ǂ	681ǂ	836ǟ	861ǟ
BP475-525	834ǟ	454*	430*	451*	1038ǟ	1070ǟ
BP500/20IR	916ǟ	888ǟ	746	686*	1133ǟ	1126ǟ
BP505-530	880ǟ	834ǟ	710	454*	1047ǟ	1095ǟ
BP530-600	680	611	505	536	545*	995ǟ
BP505-550	769	703	587	454*	550*	1033ǟ
BP560-615	809ǂ	675ǂ	558	594	738ǂ	1015ǟ
BP585-615	749ǂ	731ǂ	609	649ǂ	800ǂ	1037ǟ

Table 7 Color (0-255) average, mode, and pixel counts recorded by Image J for the binary image.

Variables	Polarized Binary Image
Mean	66.45
Mode	0
White (coded 0)	969162
Black (coded 255)	341558
Total count	1310720

Table 8 Color (0-255) average and mode recorded by Image J for the red (r), green (g), and blue (b) channels for the unaltered color image.

Variables	Polarized RGB Image	Confocal RGB Image
rMean	70.45	46.45
gMean	13.95	56.57
bMean	0.03	36.34
rMode	0	37
gMode	0	48
bMode	0	32

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Appendix 1 Samples used for each section of study.

Section of Study	Species Used	Number of Specimens	Number of Slides Made/Used
Staining, bleaching, and confocal protocols			
Staining unbleached specimens	*Acer saccharinum*	1	4
Bleaching	*Juglans nigra*	1	4
Staining bleached specimens	*J. nigra*	1	4
Imaging samples with the CLSM	*A. saccharinum* *J. nigra*	2	12
Using herbarium specimens (the effects of safranin on a CLSM image)	*A. sacccharum*	1	2
Extracting data from images	*J. nigra*	1	1

CHAPTER 3: A COMPARATIVE STUDY BETWEEN BASSOON RESONANCE WOODS AND A KNOWN NON-RESONANT WOOD

Introduction

Wood can have either a subtle or substantial effect on instrument timbre, the characteristic sound of an instrument (Holt 1990), and choosing resonant wood can be difficult because of its complex structure (Chaffney 2002). Straight grain, defect-free wood, the ability to be turned thinly on a lathe, and the ability to hold a screw are qualities that are traditionally used for choosing wood for musical instruments (Heckel and Heckel 1931). Physicists and engineers have included density and elasticity to the list of characters, based on violin studies. However, these characteristics are not foolproof, causing a lot of waste during the manufacturing process. Fox Products constructed a bassoon from a wood containing all these characteristics, *Juglans nigra* (black walnut), and the instrument projected its sound poorly in a concert hall setting. From this, it can be seen that the characters used now are not descriptive enough. Finding more accurate characters will help reduce waste of precut timber, save money during the manufacturing process, and lessen the need to cut down so many trees to find one piece of resonant wood. Also, creating a more detail description of bassoon resonant wood will help with finding suitable alternatives for the bassoon resonant woods used now. The availability of quality timber is decreasing because of pestilence and disease. American elm (*Ulmus americana*) populations were decimated by Dutch elm disease during the 20th century (Karnosky 1979), and American chestnut (*Castanea dentata*) populations were destroyed by blight at the same time (Anagnostakis 1987). Ash (*Fraxinus* spp.) have been damaged by the emerald ash borer, and maple (*Acer* spp.) have been damaged by the Asian hornedbeetle (Gandhi and Herms 2010). This and climate change are making the exclusivity of using maple for the bassoon dangerous for future instrument production (Dukes, *et al.* 2009).

Bassoons have been in existence for at least 300 years (Grove, *et al.* 1980), and one of the problems bassoon manufacturers have encountered is producing an instrument that plays in tune. This problem has mostly gone away with the advent of jointing (Langwill 1971), but other circumstances can cause a bassoon to play out of tune. Wood with defects (e.g. knots, spiral grain) cannot be cut easily and uniformly to match the surrounding defect-free wood (Hoadley 2000), which caused any instrument made with this wood to be out of tune. Tuning problems are not examined, because many scientists are more interested in timbre. Timbre studies began when

physicists and engineers started to look for more descriptive mechanical characters in the violin woods. The best descriptive characters were density (specific gravity) and elasticity (Fletcher and Rossing 1998). The most commonly used luthier woods in Europe and North America, *Picea glauca* (white spruce) and *P. sitchensis* (Sitka spruce), have a specific gravity of 0.45 and 0.42 respectively (United States Forest Products Laboratory 1974) and are classified as medium density woods. Elasticity is described by modulus of elasticity (MOE), a measure of material stiffness (Basu 2000). Materials with high MOE do not absorb much energy from an external force, and a material with a low MOE absorbs more energy. The MOE of *P. glauca* and *P. sitchensis* are 9.86 GPa and 10.8 GPa respectively (United States Forest Products Laboratory 1974), giving the woods a moderate MOE. Researchers such as Wegst (2006) and Holz (1996) used these violin wood characters to determine what woods were resonant for woodwinds. They found that any wood with a density and elasticity at least as high as white spruce would work for manufacturing bassoons, because wood does not affect a woodwind's timbre like it would a violin.

The role of material on a woodwind's timbre has been studied sporadically. Backus (1964) tested the effects of wall material on sound production using metal, plastic, and wooden clarinets. He found the material did not play a significant role in timbre but did vibrate as the instrument was being played. During this same study, he found that material did not affect timbre in a bassoon. The subtle effects on projection, blending, and intonation were not tested. This was the last time bassoon wall material was studied scientifically. Testing would now be left to instrument manufacturers and musicians.

Fox Products Corporation, a double reed instrument manufacturer in Indiana, produced a black walnut (*Juglans nigra*) bassoon to see if black walnut could be an alternative to maple (*Acer* spp.) – the most commonly used bassoon resonant wood; however the bassoon did not work (Owen, *pers. comm.*). The black walnut bassoon played well in the work room; however, when played on stage, its sound did not project to the back of the concert hall. Using the current descriptive characters of resonant woods as a guide, it should have worked. *Juglans nigra* (black walnut) is a ring-porous wood native to the Eastern Deciduous Forest, like maple. Its specific gravity and MOE are similar to maple. Both are prized woods for high quality furniture and share all the qualities traditionally looked for in a resonant wood. The characters developed from 300 years of work and the violin wood studies were not enough to distinguish *J. nigra* as a non-resonant wood. A clue may be found in the manufacturing process.

The bassoon manufacturing process, particularly the shaping portion, exposes certain characteristics of the wood to vibration, which in turn can affect how wood influences sound. Resonant timber is drilled longitudinally through the transverse face to create a bore. This bore exposes all the longitudinal faces – tangential, radial, and a series of intermediate faces called the 'cross-grain' – to the vibrating column of air within the instrument body. The most prevalent face is the 'cross-grain'. If the tangential face is considered to be the x-axis and the radial face the y-axis on a simple graph, the 'cross-grain' would occupy every angle between 0 and 90, and each angle would be unique physically. The 'cross-grain' angles greater than 45° would contain increasingly more longitudinally cut parenchyma and those angles less than 45° would contain increasingly more horizontally cut parenchyma. A study done on arch angle in a violin sound board (Schleske 1990) showed were limits on 'cross-grain' angle before dampening, or sound muting, occurred. This study is relevant to a bassoon bore, because both carving and drilling exposes all the longitudinal faces, and carving the violin sound board simulates a sharply curving growth ring. The study also demonstrates what has been found during the bassoon manufacturing process – there are limits to growth ring curvature before dampening occurs. For the marimba, it was found that dampening was correlated with the increasing amount of axial parenchyma in the woods used in this study (Brancheriau, *et al.* 2006). C. W. Bond (1976) thought vessel diameter, vessel distribution, the contents of the cells, the percentage of parenchyma, ray size, fiber volume, and fiber length should be important descriptive characters for violin resonant wood. Spycher *et al.* (2008) found that small cells would be ideal for violin soundboards. An anatomical study on bassoon woods has not been done to see if parenchyma is also a source of dampening.

Can anatomical characters be used to select resonant bassoon woods? Since both the traditional and mechanical characteristics are too similar to distinguish resonant and non-resonant bassoon wood, a logical step would be to look at the wood anatomy. Dimensional anatomy shows differences between *J. nigra* and the known bassoon resonant woods (*Acer* spp., *Dalbergia melanoxylon*, and *Pyrus communis*). The wood of *J. nigra* is also easier to shape than the resonant woods, which may have to do with the polymer distributions within the walls of each cell type. As in a rapidly growing sapling, the larger cells in the non-resonant wood may have a looser wall structure with more hemicellulose and lignin than the smaller celled resonant woods. Lignin and hemicellulose have a low MOE (Bucur 2006), causing these large cells absorb to more energy from the vibration. Consequently, any cell with a large amount of cellulose compared to lignin and hemicellulose will have a high modulus of elasticity and will not absorb energy from the vibration.

To test this question, non-resonant and resonant woods were compared using dimensional cell characteristics (length and width of each cell type, density) and wall polymer deposition (concentration of cellulose, lignin, pectin, and other aromatics produced from respiration in the lumen and wall of each cell type), and the data obtained were tested for significance between the resonant and non-resonant groups with MANOVA and ANOVA.

Materials and Methods

To determine if anatomical characters separate the resonant from non-resonant bassoon woods, dimensional (length, width, density) and cell wall characters (crystalline cellulose and lignin, pectin, and other aromatics produced from respiration exposed by sectioning) were measured and analyzed from section and maceration (dissolved wood) slides. All aromatic compounds within the cell wall were considered, because they could affect vibration in a manner similar to how a change of medium affects a wave, altering the path of the wave by refraction (Benade 1990). Specimens were obtained in slide form from GH (Gray Herbarium), MAD (University of Wisconsin – Madison Herbarium), and MU (Miami University Herbarium). These specimens were supplemented by slides created from purchased pen blanks, core and chip samples collected in the eastern Oklahoma State Parks, and wood samples donated by members of the International Wood Collectors Society (IWCS). Pen blanks were chosen because the wood quality needed to turn the wood on a microlathe, a miniature lathe used to create the body of homemade pens, is similar to that of resonant wood. Altogether eight species (hard maple encompassing two species and soft maple encompassing two species) were used in this study (Table 1) –African blackwood (*Dalbergia melanoxylon*), black walnut (*Juglans nigra*), hard maple (*Acer nigrum, A. saccharum*), mountain maple (*A. pseudoplatanus*), pear (*Pyrus communis*), and soft maple (*A. rubrum, A. saccharinum*). All species except *J. nigra* have been used as resonant bassoon woods for the last 300 years (Zadro 1975a, b).

Section slides were created by sectioning each sample face (transverse, tangential, radial, 'cross-grain') to 10 µm using an AO 860 sliding microtome. The sections were then permanently mounted with Permount (Permount™, Fisher Scientific) unstained. Maceration slides containing loose cells were created by macerating, or dissolving the connection between cells, a portion of the sample with a 1:4:5 solution of hydrogen peroxide: distilled water: glacial acetic acid (Ruzin 1999). The macerated tissue was then dried to an adhesive coated slide, stained for five minutes with a 0.01% solution of safranin (Safranin O, Fisher Scientific), and permanently mounted with

Permount (Permount™, Fisher Scientific). In total, 210 section slides and 167 maceration slides were created and imaged in this study (Appendix 1).

To collect dimensional and cell wall variable data, the slides were imaged with a compound light microscope (LM) and a confocal laser scanning microscope (CLSM). Both stained and unstained section slides were imaged using polarized and unpolarized light at 40X with a Nikon Alphaphot YS LM for measuring cell dimensions and the amount of crystalline structure (i.e. crystalline cellulose, crystals in axial parenchyma) exposed by face. To determine the amount of aromatic compounds (i.e. lignin, pectin, etc.) exposed by face, the unstained section slides were imaged on a Zeiss LM510 CLSM at 40X using a 488 nm ArNe laser and LP 475 and LP 530 filters. Settings for the CLSM (i.e. pinhole, detector gain, amplifier gain, etc.) were kept constant to record intensity of the autofluorescence. Stained section slides were not used for this, because many herbarium slides are stained only with safranin, which stains both cellulose and lignin equally and overwhelms the autofluorescence of the aromatics under laser light. To measure the cell dimensions obscured by sectioning, the maceration slides were imaged at 4X using an AmScope LM.

All the data were collected using Image J, a public domain image analysis program from the National Institute of Health (Rasband 1997-2011). Crystalline surface and aromatic polymer exposure data were collected using a color histogram created from images containing three channels – red, green, and blue. The polarized light illuminated all the crystalline structures within the wood, and most of the illumination was held in one channel when the polarized image was broken up into channels. When the confocal images were broken up by channel, the autofluorescence emitted by lignin and the aromatic by-products produced by the tree during its lifetime were delineated by region – the cell walls for the lignin and the lumina of the parenchyma and some vessels for the aromatic by-products. The crystalline surface exposure data were compiled from the red channel, which contained the majority of the crystalline fluorescence. The largest number of pixels in the color level indicated by the mode were subtracted from the total number of pixels in the image, then the result was divided by the total number of pixels in the image. For the aromatic polymer exposure data, information was collected in a similar manner using the red and blue channels. The red channel contained the majority of cell wall autofluorescence, and the blue channel held most of the cell content autofluorescence. Because some slides did not contain a 'cross-face' section, have a companion maceration slide, or were

stained permanently with safranin, data were broken up into four sets – dimensional section data, dimensional maceration data, confocal data, and polarized light data.

The datasets were analyzed in R, a free statistical software package (R Development Core Team 2008). Datasets were all checked for outliers, normality, and independence. Outliers where revealed by plotting the ordered squared Mahalanobis distances (MD) of the data points against the empirical distribution function of MD^2. Normality was verified with a Q-Q plot. Independence of the variables was confirmed using paired scatterplots. Of all the datasets, only the section data needed to be transformed using a $\log_{10}$ function. MANOVA tests were then generated using wood type (resonant and non-resonant) as the dependent factor to determine if the known resonant woods are statistically different from *Juglans nigra* (black walnut) when all the variables measured were considered. ANOVAs were generated automatically based on a single variable using type as the dependent factor to determine if the known resonant woods are statistically different from *Juglans nigra* (black walnut) using one character. MANOVA and ANOVA take the variability within a test group and compare it to the variability between test groups. If the variability within a test group is greater than the variability between test groups, then groups are not considered to be statistically different. Because of the results of the polarized light data MANOVA, a two-sample t-test was carried out on each face pair (transverse-tangential, transverse-radial, transverse-'cross-grain', tangential-radial, tangential-'cross-grain', and radial-'cross-grain') without regards to wood type. Significance for all the tests, MANOVAs and ANOVAs included, was set at $\alpha = 0.05$.

Results

MANOVA and ANOVA were used to determine if the resonant woods were statistically different from the non-resonant *Juglans nigra* (black walnut). The cell wall and dimensional observations were separated into datasets (section, maceration, confocal, and polarized light), because not all the samples used in the analyses could be measured with each microscope or had a macerated version of the specimen. Because some of the resonant woods are tropical, the temperate wood data were further separated and analyzed to determine if the tropical woods were skewing the results. Some of the woods are also ring-porous, and those woods were also analyzed separately to determine if they could also skew the results, which would give a false p-value (Table 2). MANOVA showed the resonant woods to be statistically different from the non-resonant *Juglans nigra* (black walnut) when all the variables used in the study were considered together. ANOVA showed the resonant

woods to be statistically different from the non-resonant *Juglans nigra* (black walnut) when the variables used in the study were considered individually.

Section data. Resonant wood was statistically different from the non-resonant *Juglans nigra* (black walnut) when the dataset was analyzed with MANOVA (Table 3). The results were the same when the dataset was broken up into temperate species only and ring-porous species only. When the data were analyzed with univariate ANOVA, all the variables used in the study separated the resonant wood from the non-resonant *Juglans nigra* (black walnut) in the overall data. All the variables, excluding the number of rays, separated the resonant wood from the non-resonant *Juglans nigra* (black walnut) in the temperate species data. All the variables, excluding the miminum vessel width *in situ*, separated the resonant wood from the non-resonant *Juglans nigra* (black walnut) in the ring-porous data (Table 4).

Looking at the images themselves, *Juglans nigra* and *Dalbergia melanoxylon (*African blackwood*)* have large vessels, and the axial parenchyma is more prominent and larger in *J. nigra* (Figure 1). The numbers of rays and vessels were greater in the resonant woods, and the minimum and maximum vessels widths *in situ* were greater in the non-resonant *Juglans nigra* (black walnut) samples (Table 5).

Maceration data. Resonant wood was statistically different from the non-resonant *Juglans nigra* (black walnut) when the dataset was analyzed with MANOVA (Table 3). The results were the same when the dataset was broken up into temperate species only and ring-porous species only. When the data were analyzed with univariate ANOVA, all the variables used in the study separated the resonant wood from the non-resonant *Juglans nigra* (black walnut) overall, when the temperate species were examined alone, and when the ring-porous species were examined alone (Table 4).

Examining the images before measurement, *Juglans nigra* (black walnut) had larger and longer vessels and fibers than the other species studied (Figure 2). Macerated fiber and vessel lengths were greater in the non-resonant *Juglans nigra* (black walnut) samples. The macerated vessel width was also greater in the non-resonant *Juglans nigra* (black walnut) samples (Table 5).

Polarized light data. Resonant wood was statistically different from the non-resonant *Juglans nigra* (black walnut) when the dataset was analyzed with MANOVA (Table 3). The results were the same when the dataset was broken up into temperate species only, but not when the dataset was

broken up into ring-porous species only. When the datasets were analyzed with univariate ANOVA, only the transverse face separated the resonant wood from the non-resonant *Juglans nigra* (black walnut) in the overall and temperate species datasets. None of the variables separated the resonant wood from the non-resonant *Juglans nigra* (black walnut) in the ring-porous dataset (Table 4). A two-sample t-test was used to examine the faces for statistical differences. All faces were different, except the transverse and tangential faces in the ring-porous data. All faces were different overall, except the tangential and radial faces. All faces were different when examining the temperate species alone and examining the ring-porous species alone (Table 6).

Examining the images before measurement, the tangential and 'cross-grain' faces were the brightest under polarized light, and there was no discernible difference among the species (Figure 3). The percentage of the crystalline surface exposed was greater in the transverse, radial, and tangential faces in the resonant species, and the percentage of the crystalline surface exposed was greater in the non-resonant *Juglans nigra* (black walnut) samples (Table 5).

Confocal data. Resonant wood was statistically different from the non-resonant *Juglans nigra* (black walnut) when the data were analyzed with MANOVA (Table 3). The results were the same when the temperate species were considered by themselves and when ring-porous species were considered by themselves. When the datasets were analyzed with univariate ANOVA, all the variables used in the study (except percentage of parenchyma autofluorescence – 'cross-grain', percentage of parenchyma autofluorescence – radial, percentage of wall autofluorescence – tangential, and percentage of parenchyma autofluorescence – transverse) separated the resonant wood from the non-resonant *Juglans nigra* (black walnut) in the overall data. Only the variable percentage of parenchyma autofluorescence – tangential separated the resonant wood from the non-resonant *Juglans nigra* (black walnut) in the temperate species dataset. The percentage of wall autofluorescence – 'cross-grain', percentage of wall autofluorescence – radial, and percentage of wall autofluorescence – transverse separated the resonant wood from the non-resonant *Juglans nigra* (black walnut) in the ring-porous data (Table 4).

Examining the images before measurement, *Juglans nigra* (black walnut) and *Dalbergia melanoxylon* (African blackwood) fluoresced with the greatest intensity (Figure 4). The percentage of wall autofluorescence was greater in the resonant samples for all the faces examined. The percentage of parenchyma autofluorescence was greater in the resonant samples for the radial,

tangential, and 'cross-grain' faces. Percentage of parenchyma autofluorescence was greater in the non-resonant *Juglans nigra* (black walnut) samples for the transverse face (Table 5).

Discussion

Analyses of the section, maceration, and confocal data showed there were significant differences between resonant and non-resonant wood no matter the zone (temperate or tropical) where the species are grown, or the porosity of the timber (diffuse- or ring-porous). Maximum vessel width *in situ*, axial parenchyma width, fiber length *macerated*, vessel length *macerated*, and vessel width *macerated* were larger in the non-resonant wood. Concentration of autofluorescence in the cell wall by the aromatics also distinguished the resonant from the non-resonant wood, depending on the face studied. Analysis of the polarized light data showed there was a significant difference between resonant and non-resonant wood only among the temperate species. When examining the wood solely by face, all the faces were significantly different from each other. This could be explained by the relationship among the cell wall polymers, cell type wall construction, and the effect of the polymers on the modulus of elasticity (MOE) and dampening.

Fibers of the species studied had the thickest walls of all the cell types and, from looking at the polarized light images, had a large amount of cellulose. The walls of both parenchyma and the vessels in temperate wood were of equal thinness in this study. However, the differences between the two were more easily seen under polarized light and with the CLSM. Parenchyma had a large amount of lignin (with low MOE) according to the confocal imagery and little cellulose (high MOE) according to the polarized light images, suggesting that wood containing large amounts of this cell type would be a sink for energy. Vessels, on the other hand, had less cellulose than the fibers and more than the parenchyma. It had as much aromatics as the parenchyma, suggesting the vessel would be less of a sink as the parenchyma but more of a sink as the fibers. Considering all this, the most resonant cell type would be the fiber, followed by the vessel. The parenchyma would be the least resonant cell in wood. Overall, the most resonant woods would have a low concentration of parenchyma, a high concentration of fibers and vessels; however, how a cell is cut will also play a role in resonance.

The cut of the cell is also important, because cell walls are created in layers and each layer exposes different amounts of the wall polymers. The cuts parallel to the vertical axis of growth in all the cells exposed more lignin and other aromatics than cellulose, making any parallel cut a sink to

energy. Non-resonant wood has more parallel cut cells, due to copious amounts of axial parenchyma in both the tangential and radial faces. Differences in the temperate species in the polarized light data can be explained by the tighter architecture, because of the larger number of cells in the resonant woods. Temperate resonant woods have smaller cells and can fit many more cells into a finite space. As a result, more cell wall is exposed, and if the cell is cut perpendicular to the vertical growth axis, more cellulose is exposed. Since cellulose has a MOE compared to lignin and hemicellulose, these cuts are less likely to absorb energy from the vibration and dampen sound. Differences between the resonant and non-resonant can be seen only in the transverse face, because it has the most perpendicular cuts of any face. This is corroborated by the near significance of the tangential face, which has the second largest amount of perpendicular cuts. Theoretically, the transverse face is the most resonant, followed by the tangential, then the radial. In reality, however, this theory does not stand up to scrutiny.

Members of the xylophone family use the radial face as a strike surface, and the violin family uses the radial face as the resonator surface. No instrument uses the transverse face, because the face has structural weaknesses along the growth rings, where the smaller, thick-walled cells of the late season growth meet the larger, thin-walled cells of the early season growth. The transverse face could potentially break apart with enough force. The reason for radial face preference could lie in the fiber cell wall and the openness of the face. The cut where the radial face is exposed, known as quartersawn, is reputed to be the most stable of the cuts. Even though the radial face is cut parallel to the vertical axis of growth, it is highly luminescent in polarized light, indicating a large amount of cellulose is exposed. Any face with a large amount of cellulose will have a high MOE. Also, the radial face has no voids in it, suggesting open space can be detrimental to resonance. Based on this, the order of resonance for the faces would be radial, tangential, and then transverse.

The cells comprising the faces are also important to consider. There are three studies on resonant wood that examined anatomy, one using the marimba as the instrument of concern and the other two using the violin. A study by Brancheriau et al. (2006) on marimba bars determined which anatomical characters best described resonant wood, and their findings were similar to those found in this study. They found that the amount of axial parenchyma in a wood, the number of parenchyma rays, their width and length, and fiber length and type were important characters for describing the best wood to use in marimba bar manufacture. The best woods for marimba were found to be tropical woods with little axial parenchyma, small rays, short fibers, and a homogenous

fiber type. Even though temperate woods are more prominent in bassoon manufacture than tropical woods and are more variable in structure, these characters agree with what was found for good bassoon resonant woods. C. W. Bond (1976) put forth a list of anatomical characters that might be important for the resonance of violin tone wood. He thought vessel diameter, vessel distribution, the contents of the cells, the percentage of parenchyma, ray size, fiber volume, and fiber length would be found to be important characters. Many of these characters were found to be important to both the bassoon and marimba. Spycher *et al.* (2008) found the anatomy of the ideal violin soundboard contained small cells, a finding similar to what was found in this study. The three instruments – the bassoon, the violin, and the marimba – each use their material in a different way to produce sound; yet their resonant wood share the same anatomical characters, suggesting there are base anatomical characters for all resonant wood regardless of the instrument.

Anatomical characteristics are excellent features for describing bassoon resonant wood. Axial parenchyma width, fiber length, vessel length, and vessel width *in situ* and from macerated wood separated the resonant from the non-resonant wood, whereas the mechanical properties did not. The best woods for making bassoons have small axial parenchyma, fibers, and vessels. Combining the anatomical features with the established characters used now for delineating resonant wood – traditional and mechanical characters – will reduce waste during the initial process of choosing the timber from which to construct a bassoon, because all those species that are anatomically different yet mechanically similar (i.e. *Juglans nigra*) to the established resonant woods (i.e. *Acer* spp.) will be eliminated before harvest. Further work on the mechanical properties of wood cells need to be done, in order to explain why one face (tangential, radial, or 'cross-grain') and cut (parallel or perpendicular to the vertical axis of growth) are more resonant than the others. Closer examination of the 'cross-grain' faces also needs to be completed, in order to determine the limits of a curving growth ring in regards to resonance.

Acknowledgements

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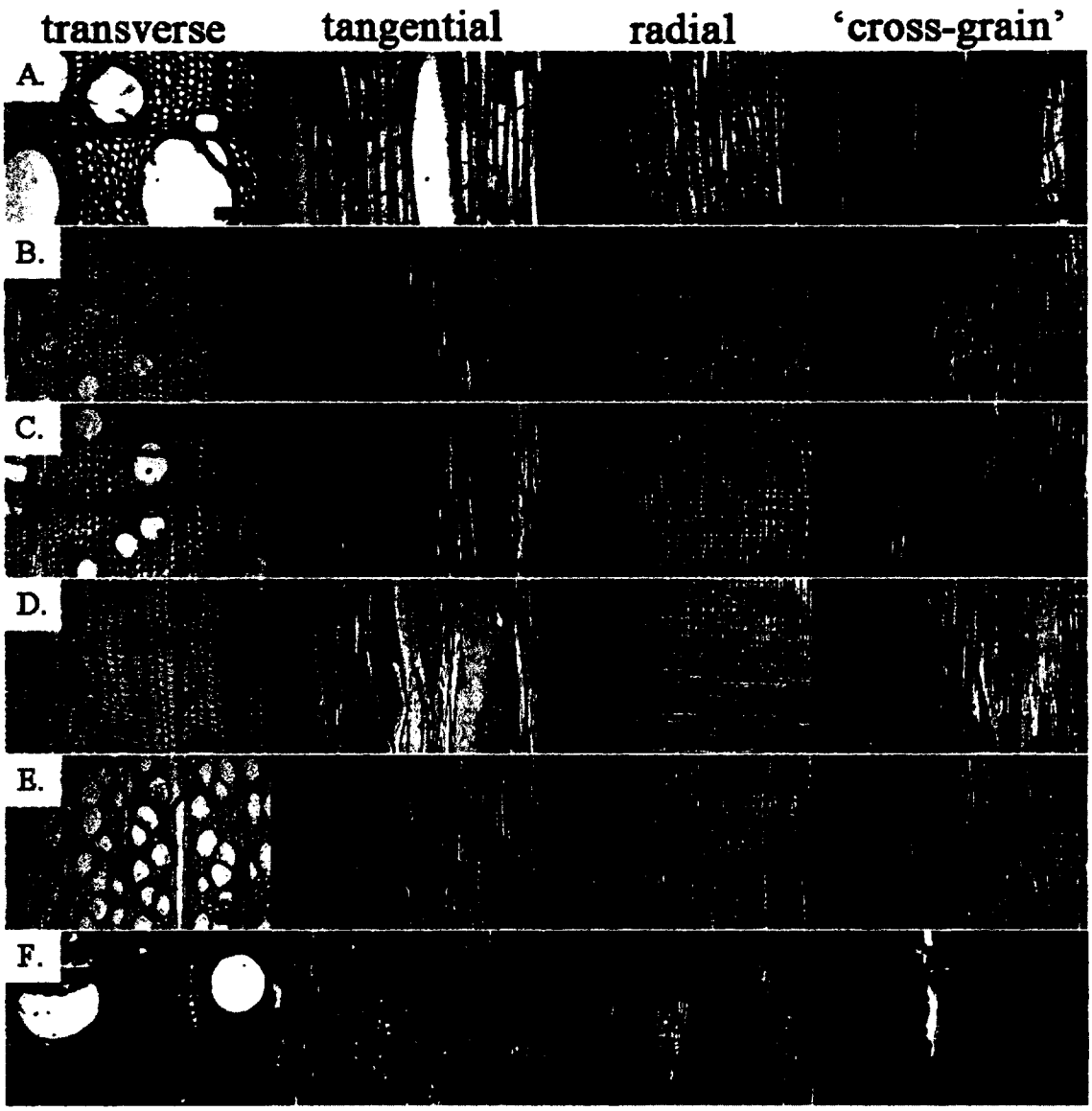

Figure 1 Light microscopy images of wood sections used in this study, taken at 40X. A. black walnut (*Juglans nigra*), B. hard maple (*Acer nigrum, A. saccharum*), C. mountain maple (*A. pseudoplatanus*), D. soft maple (*A. rubrum, A. saccharinum*), E. pear (*Pyrus communis*), F. African blackwood (*Dalbergia melanoxylon*). Scale bar = 100 µm.

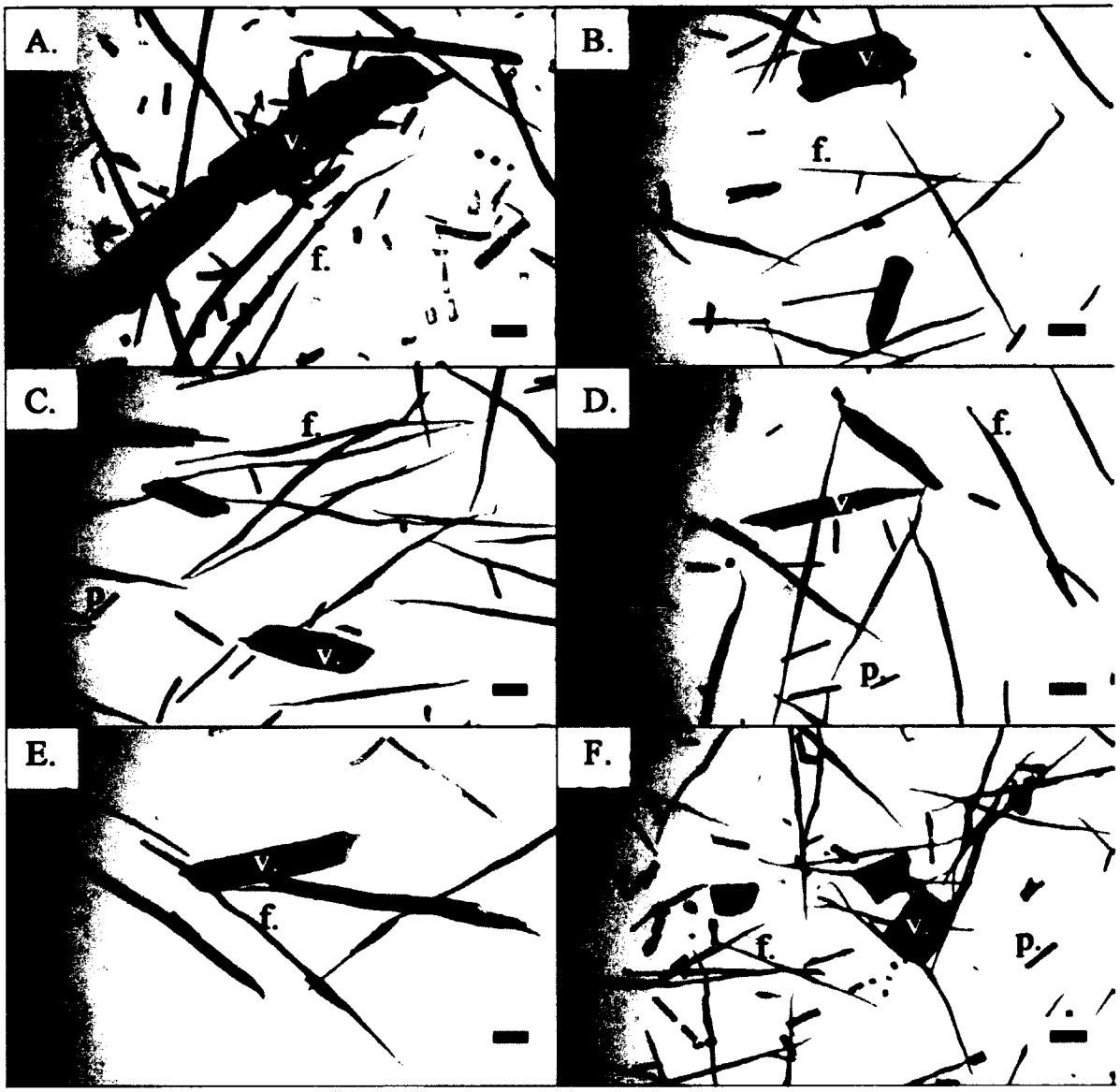

Figure 2 Light microscopy images of macerated wood used in this study, taken at 4X. A. black walnut (*Juglans nigra*), B. hard maple (*Acer nigrum, A. saccharum*), C. mountain maple (*A. pseudoplatanus*), D. soft maple (*A. rubrum, A. saccharinum*), E. pear (*Pyrus communis*), F. African blackwood (*Dalbergia melanoxylon*). f. libriform fiber, p. parenchyma, v. vessel. Scale bar = 100 μm.

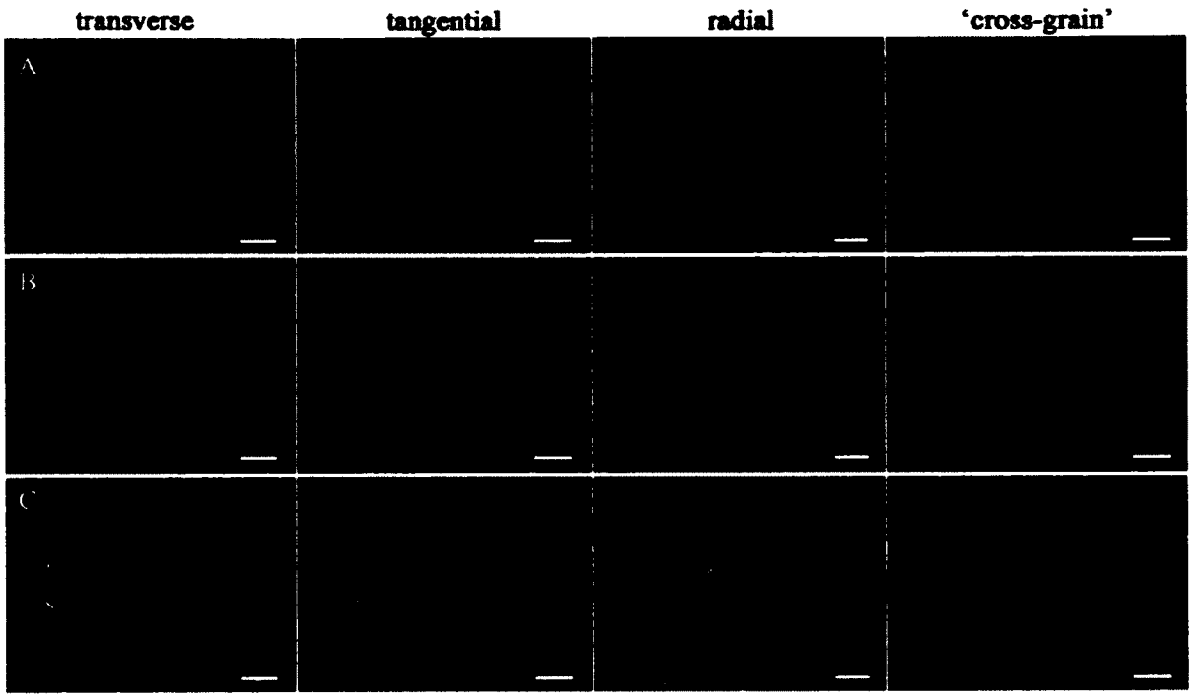

Figure 3 Light microscopy images representing the three major wood groups used in this study, taken under polarized light at 40X. A. non-resonant temperate (*Juglans nigra*), B. resonant temperate (*Acer* spp., *Pyrus communis*), C. resonant tropical (*Dalbergia melanoxylon*). Scale bar = 50 µm.

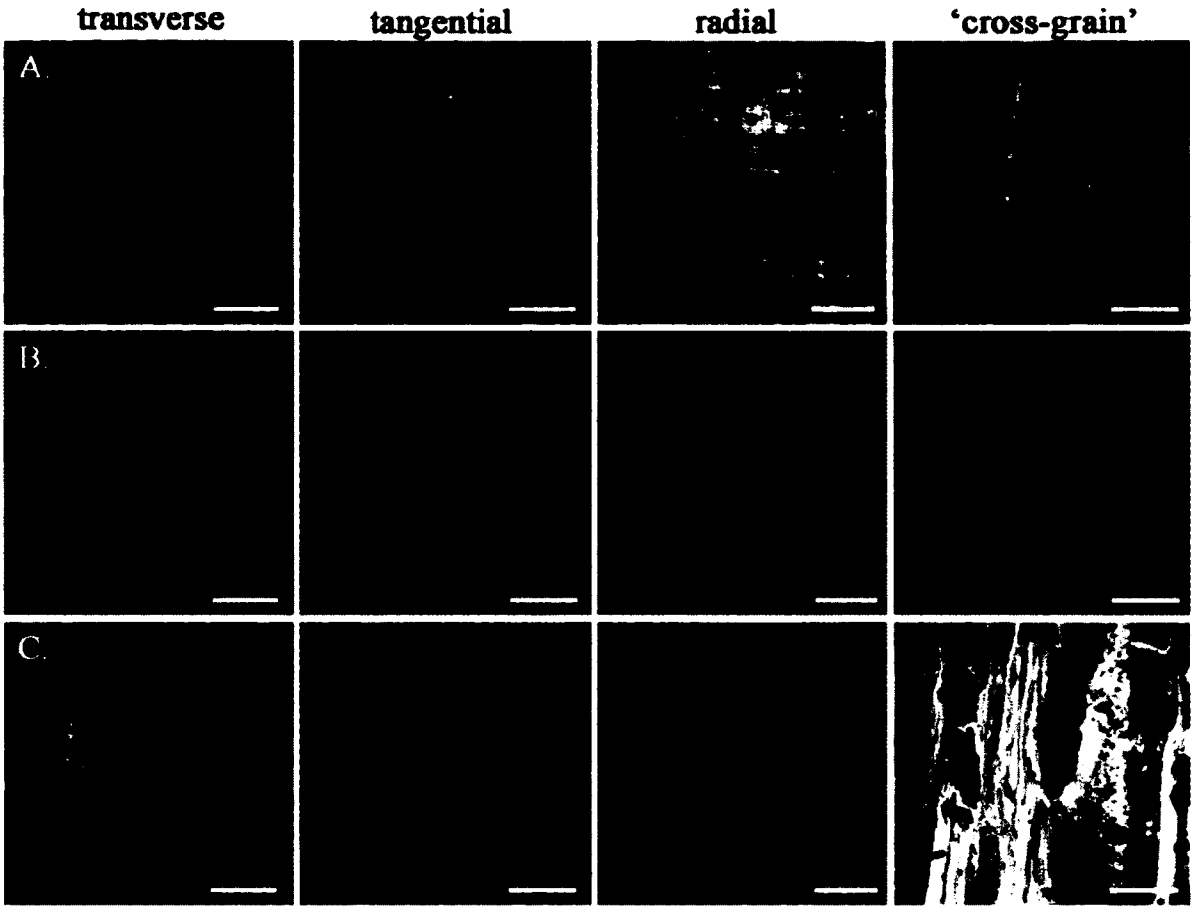

Figure 4 Confocal microscopy images representing the three major wood groups used in this study, taken with 488 nm ArNe laser at 40X. A. non-resonant temperate (*Juglans nigra*), B. resonant temperate (*Acer* spp., *Pyrus communis*), C. resonant tropical (*Dalbergia melanoxylon*). Scale bar = 50 μm.

Table 1 Origins of wood samples used in study.

Name	State/Country Collected	Wood Supply Company	Number of Trees Used in Study
mountain maple (*Acer pseudoplatanus*)	England, Spain	Fox Products Corporation	4
soft maple (*Acer rubrum, Acer saccharinum*)	Canada; USA – Arkansas, Illinois, Indiana, Missouri, New Hampshire, Kentucky, Oklahoma, Ohio, Pennsylvania, Washington, D.C.	Fox Products Corporation, Thompson Maple Products	48
hard maple (*Acer nigrum, Acer saccharum*)	USA – Arkansas, Georgia, Indiana, Oklahoma, Vermont, Virginia, Wisconsin	Fox Products Corporation, Gilmer Wood Company, Woodcraft.com, Thompson Maple Products	30
African blackwood (*Dalbergia melanoxylon*)	South Africa, Zimbabwe	Fox Products Corporation, Gilmer Wood Company, Woodcraft.com, Logs to Lumber, WoodTurningz.com	13
black walnut (*Juglans nigra*)	USA – Indiana, Iowa, Maryland, Michigan, North Carolina, Ohio, Pennsylvania, Texas, Virginia, Wisconsin	Superior Hardwood, Woodcraft.com	23
pear (*Pyrus communis*)	England; USA – Michigan	WoodTurningz.com	4

Table 2 Categorization of species used in study by wood coloration, zone of habitation, and porosity.

Species	Color of wood	Zone inhabited	Porosity
Acer nigrum (hard maple)	blond	temperate	diffuse
Acer rubrum (soft maple)	blond	temperate	diffuse
Acer saccharinum (soft maple)	blond	temperate	diffuse
Acer saccharum (hard maple)	blond	temperate	diffuse
Dalbergia melanoxylon (African blackwood)	black	tropical	ring
Juglans nigra (black walnut)	brown	temperate	ring
Pyrus communis (pear)	blond	temperate	diffuse

Table 3 Results of MANOVA carried out on anatomical data by type (resonant or non-resonant, df = 1) separated by zone (temperate or tropical) and porosity (ring- or diffuse-porous). Significance set at $\alpha = 0.05$. ‡ designates $\log_{10}$ transformation on data. α = the boundary for statistical significance for observations in a dataset, N = number of observations, Df = degrees of freedom (N-1), num Df = numerator degrees of freedom, den Df = denominator degrees of freedom, F (F-test statistic) = explained variance divided by unexplained variance, p (p-value) = the probability of getting an extreme test statistic from an observation, assuming the null hypothesis is true (Heath 1995).

Datasets	N	num Df	den Df	F	p
section ‡					
all	209	5	204	91.8	<0.0001
temperate species	166	5	161	248.3	<0.0001
ring-porous species	121	5	116	70.2	<0.0001
maceration					
all	166	3	163	295.2	<0.0001
temperate species	127	3	124	219.2	<0.0001
ring-porous species	105	3	102	228.7	<0.0001
confocal					
all	149	8	141	13.7	<0.0001
temperate species	111	8	103	13.6	<0.0001
ring-porous species	99	8	91	19.3	<0.0001
polarized light					
all	155	4	151	6.9	<0.0001
temperate species	116	4	112	13.69	<0.0001
ring-porous species	102	4	98	1.49	0.2

Table 4 Results of ANOVAs generated after MANOVA on anatomical data by type (resonant or non-resonant, df = 1) separated by zone (temperate or tropical) and porosity (ring- or diffuse-porous). Significance set at α = 0.05. ‡ designates $\log_{10}$ transformation on data. α = the boundary for statistical significance for observations in a dataset, N = number of observations, Df = degrees of freedom (N-1), num Df = numerator degrees of freedom, den Df = denominator degrees of freedom, F (F-test statistic) = explained variance divided by unexplained variance, p (p-value) = the probability of getting an extreme test statistic from an observation, assuming the null hypothesis is true (Heath 1995).

	All			Temperate Species			Ring-porous Species		
Datasets	**N Num Df Den Df**	**F**	**p**	**N Num Df Den Df**	**F**	**p**	**N Num Df Den Df**	**F**	**p**
section ‡									
# rays	209 5 204	10.5	0.001	166 5 161	0.05	0.6	121 5 116	164.1	<0.0001
# vessels		40.6	<0.0001		74.3	<0.0001		1.2	0.02
min. vessel width *in situ*		36.6	<0.0001		108.0	<0.0001		2.6	0.1
max. vessel width *in situ*		150.9	<0.0001		217.1	<0.0001		11.8	0.0003
a. paren. width		208.3	<0.0001		260.9	<0.0001		65.5	<0.0001
confocal (autofluorescence)									
%wall – 'cross-grain'	149 8 141	5.67	0.02	111 8 103	0.091	0.8	99 8 91	31.55	<0.0001
%parenchyma – 'cross-grain'		1.68	0.2		2.17	0.2		0.18	0.7
%wall – radial		3.83	0.06		0.47	0.5		9.57	0.006
%parenchyma – radial		0.31	0.6		1.54	0.2		0.016	0.9
%wall – tangential		0.62	0.4		0.2	0.7		3.36	0.08
%parenchyma – tangential		8.37	0.007		17.53	0.0005		1.92	0.2
%wall – transverse		5.8	0.02		1.17	0.3		14.85	0.001
%parenchyma – transverse		1.41	0.2		0.72	0.4		2.09	0.2
polarized light									
transverse	155	27.1	<0.0001	116	46.82	<0.0001	102	3.7	0.06
tangential	4	2.32	0.1	4	0.85	0.4	4	2.71	0.3
radial	151	2.056	0.2	112	1.63	0.2	98	1.0	0.1
'cross-grain'		0.58	0.4		2.99	0.09		1.81	0.2
maceration									
fiber length	166 3 163	759.2	<0.0001	127 3 124	506.2	<0.0001	105 3 102	555	<0.0001
vessel length		142.02	<0.0001		62.7	<0.0001		432.1	<0.0001
vessel width		190.6	<0.0001		279.5	<0.0001		43.5	<0.0001

Table 5 Averages of variables used in studies, separated by type (non-resonant, resonant).

Variables	Non-resonant	Resonant
number of rays	6	8
minimum ray width (# of cells wide)	1	1
maximum ray width (# of cells wide)	3	3
number of vessels	4	9
minimum vessel width *in situ* (μm)	38.96	25.18
maximum vessel width *in situ* (μm)	97.74	46.82
axial parenchyma width (μm)	14.79	3.62
macerated fiber length (μm)	1161.56	624.86
macerated vessel length (μm)	478.38	248.0099
macerated vessel width (μm)	207.83	98.31
% crystalline surface exposed – transverse	69.9	78.3
% crystalline surface exposed – radial	59.1	65.0
% crystalline surface exposed – tangential	64.5	69.9
% crystalline surface exposed – 'cross-grain'	83.6	81.3
% aromatic compounds exposed – wall		
transverse	80.6	87.1
tangential	76.9	81.3
radial	64.4	74.2
'cross-grain'	62.6	80.3
aromatic compounds exposed – parenchyma contents		
transverse	72.9	66.3
tangential	57.6	68.7
radial	72.4	74.3
'cross-grain'	61.0	67.2

Table 6 Results of two-sample t-tests performed on polarized light data. Significance set at $\alpha = 0.05$. α = the boundary for statistical significance for observations in a dataset, N = number of observations, Df = degrees of freedom (N-1), t (t-statistic) = (sample average – a predetermined population average) divided by the standard error of the dataset, p (p-value) = the probability of getting an extreme test statistic from an observation, assuming the null hypothesis is true (Heath 1995).

	All			Temperate Species			Ring-porous Species		
Face Combinations	**df**	**t**	**p**	**df**	**t**	**p**	**df**	**t**	**p**
transverse vs. tangential	224.6	3.7	0.0003	168.2	4.0	0.0001	143.1	1.6	0.1
transverse vs. radial	208.3	5.6	<0.0001	152.0	4.9	<0.0001	135.2	3.8	0.0002
transverse vs. 'cross-grain'	241.7	-4.2	<0.0001	172.1	-2.5	0.01	196.6	-8.2	<0.0001
tangential vs. radial	303.7	-1.9	0.06	223.2	-1.3	0.2	201.4	-1.9	0.06
tangential vs. 'cross-grain'	305.1	-6.2	<0.0001	231.6	-5.1	<0.0001	159.9	-6.8	<0.0001
radial vs. 'cross-grain'	289.5	-7.7	<0.0001	219.8	-5.9	<0.0001	149.6	-8.4	<0.0001

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Appendix 1 List of samples used and where samples obtained.

Scientific name	O.T.U.	Location	Herbarium
Acer nigrum	ACNI5-Fox	Fox Products Corporation	
Acer nigrum	ACNI523586	n.a.	GH
Acer nigrum	MADw2858	Indiana	MADw
Acer nigrum	Y11444		MADw
Acer nigrum	MADw88663		MADw
Acer nigrum	982		MADw
Acer pseudoplatanus	ACPS-E	private collection	
Acer pseudoplatanus	ACPS-Fox	Fox Products Corporation	
Acer pseudoplatanus	Y-Rudinsky-1946		MADw
Acer pseudoplatanus	Block29	England: Suffolk	MADw
Acer rubrum	ACRU-Fox	Fox Products Corporation	
Acer rubrum	ACRU-001		
Acer rubrum	ACRUOK-002	Oklahoma: Red Rock Canyon State Park	
Acer rubrum	ACRUOK-001	Oklahoma: Greenleaf State Park	
Acer rubrum	ACRUGH	Illinois	GH
Acer rubrum	ACRU23630	n.a.	GH, MU
Acer rubrum	ACRU2827	Missouri	MU
Acer rubrum	ACRU2801	Ohio	MU
Acer rubrum	ACRU685	Pennsylvania	MU
Acer rubrum	ACRU10032	Pennsylvania	MU
Acer rubrum	ACRU10027	Pennsylvania	MU
Acer rubrum	ACRU6699	New Hampshire	MU
Acer rubrum	ACRU10034	Pennsylvania	MU
Acer rubrum	Block1177	Canada	MADw
Acer rubrum	MADwACRU		MADw
Acer rubrum	MADw971	New Hampshire	MADw
Acer rubrum	8265		MADw
Acer rubrum	MADw8769		MADw
Acer rubrum	MADw9196		MADw
Acer rubrum	MADw2812		MADw
Acer rubrum	MADw2810		MADw
Acer rubrum	MADw2811	Washington D.C.	MADw
Acer rubrum	18210	Kentucky	MADw
Acer rubrum	877		MADw
Acer rubrum	MADw8989		MADw
Acer rubrum var. *trilobum*	ACRU24625	n.a.	GH

Appendix 1 continued

Scientific name	O.T.U.	Location	Herbarium
Acer saccharinum	ACSA2-002	Oklahoma: Red Rock Canyon State Park	
Acer saccharinum	ACSA2-001	Oklahoma: Greenleaf State Park	
Acer saccharinum	ACSA2-003	Oklahoma: Mannford, OK	
Acer saccharinum	ACSA2WC001	bought from Woodcraft.com	
Acer saccharinum	ACSA2WC002	bought from Woodcraft.com	
Acer saccharinum	ACSA2WC003	bought from Woodcraft.com	
Acer saccharinum	ACSA2WC004	bought from Woodcraft.com	
Acer saccharinum	ACSA2WC005	bought from Woodcraft.com	
Acer saccharinum	MADw2835/SJRw4493 1		MADw
Acer saccharinum	MADw8743		MADw
Acer saccharinum	MADwTriarch		MADw
Acer saccharinum	MADwACSA2		MADw
Acer saccharum	ACSA3OK001/ACSA3 OK	Oklahoma: Red Rock Canyon State Park	
Acer saccharum	ACSA3-G	Gilmer Wood Company	
Acer saccharum	ACSA3WC001	bought from Woodcraft.com	
Acer saccharum	ACSA3WC002	bought from Woodcraft.com	
Acer saccharum	ACSA3WC003	bought from Woodcraft.com	
Acer saccharum	ACSA3WC004	bought from Woodcraft.com	
Acer saccharum	ACSA3WC005	bought from Woodcraft.com	
Acer saccharum	ACSA3WC011	bought from Woodcraft.com	
Acer saccharum	ACSA3WC012	bought from Woodcraft.com	
Acer saccharum	ACSA3WC013	bought from Woodcraft.com	
Acer saccharum	ACSA3WC014	bought from Woodcraft.com	
Acer saccharum	ACSA3WC015	bought from Woodcraft.com	
Acer saccharum	ACSA3WC016	bought from Woodcraft.com	
Acer saccharum	ACSA3WC017	bought from Woodcraft.com	
Acer saccharum	ACSA3WC018	bought from Woodcraft.com	
Acer saccharum	ACSA3WC019	bought from Woodcraft.com	
Acer saccharum	ACSA3WC020	bought from Woodcraft.com	
Acer saccharum	ACSA3PJM	n.a.	MU
Acer saccharum	ACSA315002	n.a.	GH
Acer saccharum	ACSA37408	n.a.	GH
Acer saccharum	ACSA3-GH	n.a.	GH
Acer saccharum	ACSA36463	Virginia	MU

Appendix 1 continued

Scientific name	O.T.U.	Location	Herbarium
Acer saccharum	ACSA36964	Vermont	MU
Acer saccharum	ACSA36969	Vermont	MU
Acer saccharum	MADwACSA3		MADw
Acer saccharum	MADw9438		MADw
Acer saccharum	MADw9394		MADw
Acer saccharum	MADw8724		MADw
Acer saccharum	32		MADw
Acer saccharum	71		MADw
Acer saccharum	MADw43143	Wisconsin	MADw
Acer saccharum	MADw26601		MADw
Acer saccharum	MADw2854		MADw
Acer saccharum	MADw8782		MADw
Acer saccharum	MADw4357		MADw
Acer saccharum	MADw26602		MADw
Acer saccharum f. *glaucum*	ACSA318063	n.a.	GH
Acer spp.	SMAPLE	Thompson Maple Products	
Acer spp.	ACRU10027	Oregon	
Acer spp.	ACEROK-001	Oklahoma: Sequoyah Bay State Park	
Acer spp.	ACEROK-002	Oklahoma: Sequoyah Bay State Park	
Acer spp.	ACEROK-003	Oklahoma: Sequoyah Bay State Park	
Acer spp.	ACEROK-004	Oklahoma: Robbers Cave State Park	
Acer spp.	ACEROK-005	Oklahoma: Sequoyah Bay State Park	
Acer spp.	ACEROK-006	Oklahoma: Robbers Cave State Park	
Acer spp.	ACEROK-007	Oklahoma: Robbers Cave State Park	
Acer spp.	ACEROK-008	Oklahoma: Robbers Cave State Park	
Acer spp.	ACEROK-009	Oklahoma: Okmulgee State Park	
Acer spp.	ACEROK-010	Oklahoma: Okmulgee State Park	
Acer spp.	ACEROK-011	Oklahoma: Okmulgee State Park	
Acer spp.	LTLHM001	Indiana: Logs to Lumber	
Acer spp.	LTLSM001	Indiana: Logs to Lumber	
Acer spp.	LTLSM002	Indiana: Logs to Lumber	
Acer spp.	LTLSM003	Indiana: Logs to Lumber	

Appendix 1 continued

Scientific name	O.T.U.	Location	Herbarium
Acer spp.	LTLSM004	Indiana: Logs to Lumber	
Acer spp.	LTLSM005	Indiana: Logs to Lumber	
Acer spp.	LTLHM002	Indiana: Logs to Lumber	
Acer spp.	LTLHM003	Indiana: Logs to Lumber	
Acer spp.	LTLHM004	Indiana: Logs to Lumber	
Acer spp.	LTLHM005	Indiana: Logs to Lumber	
Dalbergia melanoxylon	DAMELFox	Fox Products Corporation	
Dalbergia melanoxylon	DAMELG	Gilmer Wood Company	
Dalbergia melanoxylon	DAMELWC001	bought from Woodcraft.com	
Dalbergia melanoxylon	DAMELWC002	bought from Woodcraft.com	
Dalbergia melanoxylon	DAMELWC003	bought from Woodcraft.com	
Dalbergia melanoxylon	DAMELWC004	bought from Woodcraft.com	
Dalbergia melanoxylon	DAMELWC005	bought from Woodcraft.com	
Dalbergia melanoxylon	DAMELWC006	bought from Woodcraft.com	
Dalbergia melanoxylon	DAMELWC007	bought from Woodcraft.com	
Dalbergia melanoxylon	DAMELWC008	bought from Woodcraft.com	
Dalbergia melanoxylon	DAMELWC009	bought from Woodcraft.com	
Dalbergia melanoxylon	DAMELWC010	bought from Woodcraft.com	
Dalbergia melanoxylon	DAMELWC011	bought from Woodcraft.com	
Dalbergia melanoxylon	DAMELWC012	bought from Woodcraft.com	
Dalbergia melanoxylon	DAMELWC013	bought from Woodcraft.com	
Dalbergia melanoxylon	DAMELWC014	bought from Woodcraft.com	
Dalbergia melanoxylon	DAMELWC015	bought from Woodcraft.com	
Dalbergia melanoxylon	DAMELWC016	bought from Woodcraft.com	
Dalbergia melanoxylon	DAMELWC017	bought from Woodcraft.com	
Dalbergia melanoxylon	DAMELWC018	bought from Woodcraft.com	
Dalbergia melanoxylon	DAMELWC019	bought from Woodcraft.com	
Dalbergia melanoxylon	DAMELWC020	bought from Woodcraft.com	
Dalbergia melanoxylon	DAMEL19284	n.a.	GH
Dalbergia melanoxylon	DAMEL19285	n.a.	GH
Dalbergia melanoxylon	GW001	South Africa: bought from Global Woods	
Dalbergia melanoxylon	GW002	South Africa: bought from Global Woods	
Dalbergia melanoxylon	GW004	South Africa: bought from Global Woods	
Dalbergia melanoxylon	GW005	South Africa: bought from Global Woods	

Appendix 1 continued

Scientific name	O.T.U.	Location	Herbarium
Dalbergia melanoxylon	Block1644	Zimbabwe	MADw
Dalbergia melanoxylon	27502		MADw
Dalbergia melanoxylon	30011		MADw
Dalbergia melanoxylon	Shak18	Kluge Park	MADw
Dalbergia melanoxylon	LTLDAMEL001	Indiana: Logs to Lumber	
Dalbergia melanoxylon	LTLDAMEL002	Indiana: Logs to Lumber	
Dalbergia melanoxylon	DAMELWC021	bought from Woodcraft.com	
Dalbergia melanoxylon	DAMELWC022	bought from Woodcraft.com	
Dalbergia melanoxylon	DAMELWC023	bought from Woodcraft.com	
Dalbergia melanoxylon	DAMELWC024	bought from Woodcraft.com	
Dalbergia melanoxylon	DAMELWC025	bought from Woodcraft.com	
Dalbergia melanoxylon	DWT001	bought from WoodTurningz.com	
Dalbergia melanoxylon	DWT002	bought from WoodTurningz.com	
Dalbergia melanoxylon	DWT003	bought from WoodTurningz.com	
Juglans nigra	JUNISH001	Superior Hardwood	
Juglans nigra	JUNISH002	Superior Hardwood	
Juglans nigra	JUNIWC001	Pennsylvania: Woodcraft.com	
Juglans nigra	JUNIWC002	Pennsylvania: Woodcraft.com	
Juglans nigra	JUNIWC003	Pennsylvania: Woodcraft.com	
Juglans nigra	JUNIWC004	Pennsylvania: Woodcraft.com	
Juglans nigra	JUNIWC005	Pennsylvania: Woodcraft.com	
Juglans nigra	JUNIWC006	Pennsylvania: Woodcraft.com	
Juglans nigra	JUNIWC007	Pennsylvania: Woodcraft.com	
Juglans nigra	JUNIWC008	Pennsylvania: Woodcraft.com	
Juglans nigra	JUNIWC009	Pennsylvania: Woodcraft.com	
Juglans nigra	JUNIWC010	Pennsylvania: Woodcraft.com	
Juglans nigra	JUNIWC001	bought from Woodcraft.com	
Juglans nigra	JUNIWC002	bought from Woodcraft.com	
Juglans nigra	JUNIWC003	bought from Woodcraft.com	
Juglans nigra	JUNIWC004	bought from Woodcraft.com	
Juglans nigra	JUNIWC005	bought from Woodcraft.com	
Juglans nigra	JUNIWC006	bought from Woodcraft.com	
Juglans nigra	JUNIWC007	bought from Woodcraft.com	
Juglans nigra	JUNIWC008	bought from Woodcraft.com	
Juglans nigra	JUNIWC009	bought from Woodcraft.com	
Juglans nigra	JUNIWC010	bought from Woodcraft.com	
Juglans nigra	JUNIWC011	bought from Woodcraft.com	

Appendix 1 continued

Scientific name	O.T.U.	Location	Herbarium
Juglans nigra	JUNIWC012	bought from Woodcraft.com	
Juglans nigra	JUNIWC013	bought from Woodcraft.com	
Juglans nigra	JUNIWC014	bought from Woodcraft.com	
Juglans nigra	JUNIWC015	bought from Woodcraft.com	
Juglans nigra	JUNIWC016	bought from Woodcraft.com	
Juglans nigra	JUNIWC017	bought from Woodcraft.com	
Juglans nigra	JUNIWC018	bought from Woodcraft.com	
Juglans nigra	JUNIWC019	bought from Woodcraft.com	
Juglans nigra	JUNIWC020	bought from Woodcraft.com	
Juglans nigra	LL002	Michigan: Landfill Lumber	
Juglans nigra	LL003	Michigan: Landfill Lumber	
Juglans nigra	LL004	Michigan: Landfill Lumber	
Juglans nigra	LL005	Michigan: Landfill Lumber	
Juglans nigra	REE001	Virginia: The River's Edge Exotics	
Juglans nigra	REE002	Virginia: The River's Edge Exotics	
Juglans nigra	REE003	Virginia: The River's Edge Exotics	
Juglans nigra	REE004	Virginia: The River's Edge Exotics	
Juglans nigra	REE005	Virginia: The River's Edge Exotics	
Juglans nigra	REE006	Virginia: The River's Edge Exotics	
Juglans nigra	REE007	Virginia: The River's Edge Exotics	
Juglans nigra	REE008	Virginia: The River's Edge Exotics	
Juglans nigra	REE009	Virginia: The River's Edge Exotics	
Juglans nigra	REE010	Virginia: The River's Edge Exotics	
Juglans nigra	LTL001	Indiana: Logs to Lumber	
Juglans nigra	LTL002	Indiana: Logs to Lumber	
Juglans nigra	LTL003	Indiana: Logs to Lumber	
Juglans nigra	LTL004	Indiana: Logs to Lumber	
Juglans nigra	LTL005	Indiana: Logs to Lumber	
Juglans nigra	LTL006	Indiana: Logs to Lumber	
Juglans nigra	LTL007	Indiana: Logs to Lumber	
Juglans nigra	LTL008	Indiana: Logs to Lumber	
Juglans nigra	LTLJUNI001	Indiana: Logs to Lumber	
Juglans nigra	LTLJUNI002	Indiana: Logs to Lumber	
Juglans nigra	LTLJUNI003	Indiana: Logs to Lumber	
Juglans nigra	LTLJUNI004	Indiana: Logs to Lumber	
Juglans nigra	LL006	Michigan: Landfill Lumber	
Juglans nigra	LL007	Michigan: Landfill Lumber	

Appendix 1 continued

Scientific name	O.T.U.	Location	Herbarium
Juglans nigra	LL008	Michigan: Landfill Lumber	
Juglans nigra	LL009	Michigan: Landfill Lumber	
Juglans nigra	LL010	Michigan: Landfill Lumber	
Juglans nigra	LL011	Michigan: Landfill Lumber	
Juglans nigra	LL012	Michigan: Landfill Lumber	
Juglans nigra	LL013	Michigan: Landfill Lumber	
Juglans nigra	LL014	Michigan: Landfill Lumber	
Juglans nigra	LL015	Michigan: Landfill Lumber	
Juglans nigra	LL016	Michigan: Landfill Lumber	
Juglans nigra	CA001	Iowa: Cedar Antler	
Juglans nigra	CA002	Iowa: Cedar Antler	
Juglans nigra	CA003	Iowa: Cedar Antler	
Juglans nigra	CA004	Iowa: Cedar Antler	
Juglans nigra	CA005	Iowa: Cedar Antler	
Juglans nigra	CA006	Iowa: Cedar Antler	
Juglans nigra	CA007	Iowa: Cedar Antler	
Juglans nigra	CA008	Iowa: Cedar Antler	
Juglans nigra	CA009	Iowa: Cedar Antler	
Juglans nigra	CA010	Iowa: Cedar Antler	
Juglans nigra	JUNIAHP	n.a.	MU
Juglans nigra	MADw815	N. Carolina	MADw
Juglans nigra	SJRw11670	Virginia	MADw
Juglans nigra	8-a-2	Wisconsin	MADw
Juglans nigra	MADw1119	Texas	MADw
Juglans nigra	SJRw46160	Maryland	MADw
Juglans nigra	MADw8750	Ohio	MADw
Juglans nigra	MADw17370	Iowa	MADw
Juglans nigra	MADw14825		MADw
Juglans nigra	MADwJUNI		MADw
Juglans nigra	Block76	Indiana	MADw
Juglans nigra	MADw8986		MADw
Juglans nigra	MADw1935		MADw
Juglans nigra	MADw1598		MADw
Juglans nigra	Y11669		MADw
Juglans nigra	MADw811		MADw

Appendix 1 continued

Scientific name	O.T.U.	Location	Herbarium
Pyrus communis	SR34		MADw
Pyrus communis	Block1381	England: Suffolk	MADw
Pyrus spp.	PYRUSWT001	bought from WoodTurningz.com	
Pyrus spp.	LLPear001	Michigan: Landfill Lumber	
Pyrus spp.	LLPear002	Michigan: Landfill Lumber	
Pyrus spp.	LLPear003	Michigan: Landfill Lumber	
Pyrus spp.	LLPear004	Michigan: Landfill Lumber	
Pyrus spp.	LLPear005	Michigan: Landfill Lumber	
Pyrus spp.	PYRUSWT002	bought from WoodTurningz.com	
Pyrus spp.	PYRUSWT003	bought from WoodTurningz.com	
Pyrus spp.	PYRUSWT004	bought from WoodTurningz.com	
Pyrus spp.	PYRUSWT005	bought from WoodTurningz.com	

CHAPTER 4: TAPPING WOOD TO DETERMINE RESONANCE QUALITIES FOR BASSOON WOODS

Introduction

Material used to construct a musical instrument body has either a significant effect (i.e. the soundboard of a violin) or a subtle effect (i.e. the barrel of a clarinet) on an instrument's complex sound. In a violin, the material used to create the soundboard affects the timbre, which is the characteristic quality of sound produced by a particular instrument (Holt 1990). For a woodwind, especially the bassoon, the material affects how well the instruments projects to the back of the concert hall; the ease of intonation (playing the instrument in tune); and ability to blend sounds with other instruments. Even though the magnitude of effect is smaller in a woodwind, choosing the proper material is still difficult and there is a lot of waste involved. Having a system to recognize appropriate resonant woods for a woodwind like a bassoon would save time, money, and the resources of a forest being reduced in size.

Each instrument type creates a complex sound with a timbre that the listener can immediately recognize. Somewhere in the complex sound lies the frequency or frequencies that contain the timbre; however, no one is sure exactly where the timbre is (Fletcher and Rossing 1998). Human hearing ranges from 20 Hz to 20 kHz, but the lower and upper limits are more difficult to differentiate (Cutnell and Johnson 2012). Musical instruments play within the range of human hearing. Pianos have the widest range, A_0 (27.5 Hz) to C_8 (4186 Hz) (Fletcher and Rossing 1998). Bassoons, as a low instrument, have a range of $B_1{}^{\flat}$ (58.27 Hz) to $E_5{}^{\flat}$ (622.5 Hz) (Langwill 1971). Some research suggests the timbre is found in the beginning transients, the noise components that lie in upper and lower regions of a complex sound (Saldanha and Corso 1964). Where ever the timbre is, it is obvious from the ambiguity of its nature that it lies on the cusp of human hearing, and it seems reasonable that the material used to create the instrument bodies have an effect on the noise components of the complex sound.

The material chosen for woodwinds and strings is typically wood, and all the woods used have certain features or characteristics in common. Traditionally, woods chosen for instruments were straight-grained and defect-free, the wood fine-grained enough to be turned finely on a lathe and hold screws for keywork (Heckel and Heckel 1931). *Picea abies* (white spruce),a resonant wood

used particularly for stringed instruments, has a specific minimum density and modulus of elasticity (MOE), modern characters used to describe woodwind resonant wood by some researchers (Holz 1996, Wegst 2006). Stringed instrument manufacturers, sometimes known as luthiers, also employ a technique called tapping, which is rapping on a potential resonant timber to see if a clear, loud sound can be produced (Siminoff 2002). This technique is not used in woodwinds. Hutchins and Fielding (1968) correlated tapping methods of luthiers to more quantifiable scientific methods and found tapping can differentiate not only resonant and non-resonant timber, but resonant and non-resonant spots in a piece of timber. The bodies created from the resonant woods are used by the instruments to create sound in different ways.

Woodwinds and stringed instruments utilize their bodies for different purposes, and, as a result, the wood used can have either a significant or subtle effect on timbre. Reed woodwinds use their bodies to hold a constantly rotating column of air within a hollow tube, have a mouthpiece with either one moving side (i.e. the clarinet and saxophone) or two moving sides (i.e. the bassoon and oboe). The vibration is initiated by blowing into the hole created by the two sides of the mouthpiece. Holes placed along the longitudinal length of the instrument are opened or closed, which changes the length of the column, producing a specific note. The hollow tube is created by boring a hole parallel to the longitudinal faces of a timber, and the orientation of the wood affects the vibration initiated by the player, particularly in a bassoon (Benade 1990). Stringed instruments use their soundboard – the top plate of the instrument – as a resonator, and the vibration is created by plucking, bowing, or strumming a string (Grove, *et al.* 1980). Players change the length of the string or strings to produce a specific note, and the soundboard along with the cavity created by the rest of the body amplifies the vibration.

In chapter three, anatomical characters were found to be descriptive of resonant woods. There are three major cell types in hardwood (the tree forming angiosperms) – vessels, fibers, and parenchyma. Less resonant woods were found to contain long vessels and fibers and large axial parenchyma; whereas, more resonant woods had shorter vessels and fibers and little to no axial parenchyma. The amount of these cell types is different for each face, or unique side, of wood. How a face reacts to vibration depends on the proportion and cut of the cell types.

The bore of the bassoon exposes three longitudinal faces to vibration – tangential, radial, and 'cross-grain'. Each face reacts uniquely to the vibration. In relation to tree growth, the tangential

face is parallel to the vertical (longitudinal) growth, or height, and perpendicular to the horizontal (transverse) growth, or girth (Hoadley 2000). Cells in this face are longitudinally cut vessels, fibers, and axial parenchyma, as well as transversely cut ray parenchyma. The radial face is parallel to both the vertical and horizontal growth of the tree (Hoadley 2000), and the cells are arranged in two layers. In the bottom layer, there are longitudinally cut vessels, fibers, and axial parenchyma; in the top layer, the ray parenchyma longitudinally cut in bands. The 'cross-grain' face contains gradations of the radial and tangential faces in different proportions, depending on the orientation of the 'cross-grain' cut. For example, 'cross-grain' faces closer to the radial face containing more features of the radial than the tangential and *vice versa.* The cut of each cell (longitudinal or transverse) exposes more or less of the polymers used to create the cell wall, and polymers have an effect on the mechanical properties of the wood (Bucur 2006), as well as the dampening (muting) abilities of wood on an instrument's sound.

In order to create a wood cell wall (Figure 1), the polymers cellulose, lignin, and hemicellulose are arranged in layers. The cell wall is created in two layers, the primary wall and the secondary wall. Cellulose is used to create a matrix from strands of crystalline cellulose called microfibrils to which hemicellulose can attach, followed by lignin attaching to hemicellulose. In the primary wall, the cellulose microfibrils are arranged almost perpendicular to the vertical axis of the cell's growth; in the secondary wall, the microfibrils are arranged in three laminal layers – S_1, S_2, and S_3. Microfibrils in the S_1 layer are arranged at an angle of at least 60° from the vertical axis of the cell's growth. In the S_2 layer, the microfibrils are arranged 30° – 40° from the vertical axis of the cell's growth. Microfibrils in the final layer are arranged in a similar fashion as the primary cell wall (Dickison 2000, Esau 1977, Mauseth 1988). Cellulose, particularly in the S_2 layer, affects the tensile strength of a timber (United States Forest Products Laboratory 1974), yet it does not absorb energy from a force like vibration (Bucur 2006, Salmén 2004). Hemicellulose is used during cell expansion as a stabilizer, then as a binder for lignin when the cell begins lignification (Bowes and Mauseth 2008). This polymer affects compressional strength (United States Forest Products Laboratory 1974) and absorbs energy from vibration (Bucur 2006, Salmén 2004). Lignin is as an impermeable water and microbial barrier and affects the compressional strength of the cell wall (Forbes and Watson 1992). Like hemicellulose, lignin absorbs energy from vibration (Bucur 2006, Salmén 2004).

The cell wall polymers are exposed in different amounts by the direction of the wall cut and the cell type. Typically longitudinal cuts expose more hemicellulose and lignin than cellulose, whereas a transverse cut will expose more cellulose. Fibers cut either way will expose a great deal of cellulose, because fibers require thick cell wall to perform its structural function. Vessels will expose more lignin and hemicellulose in a longitudinal cut than the fiber, because its purpose is both structural and water transport and a thick wall is not needed until later in the growing season (Dickison 2000, Esau 1977, Mauseth 1988). Parenchyma cells are used for storage of waste from respiration and for lateral transport of nutrients. To perform these functions, parenchyma needs a thinner wall, which exposes more lignin by the longitudinal cut (Chafe 1974). Consequently, faces that contain more longitudinal cuts than transverse will absorb more energy from a vibration than those containing transverse cuts. These faces are exposed in differing amounts during the bassoon manufacturing process and have an effect on the resonance of the timber.

During the manufacturing process of bassoons, some woods classified as resonant suddenly become non-resonant (Owen *pers. comm.*). When the initial hole is drilled longitudinally through the timber, all the longitudinal faces are exposed. If the growth rings of the wood are straight, the 'cross-grain' faces have equivalent gradations between the radial and tangential faces. When the growth rings curve, the gradation will lean more towards one face than another and dampening will occur. A study focusing on the angle of arch in a violin soundboard found the steepness of the arch has a direct effect on dampening (Schleske 1990). Even though a violin soundboard is carved and not bored, the effect is similar to a naturally curving growth ring. Violin soundboards are oriented in such a way that the radial face is exposed to the vibrating string. When an arch is too steep, or nearly parallel to the tangential face, the 'cross-grain' faces show more tangential characteristics than radial. Similarly, in a bassoon resonant timber that has a pronounced curving growth ring, the bore will expose 'cross-grain' faces that are more similar to the tangential face. During the forming process of the bassoon, a wood classified as resonant can suddenly become non-resonant, and the wood has to be thrown away. Manufacturers have yet to find what angle the growth rings can be and still be resonant (Owen *pers. comm.*). To discard any wood with the slightest curve in its growth ring would greatly limit the supply of timber and would not be cost-effective. Face orientation and cell wall cut is not accounted for in the resonant wood definition, making the definition of resonant wood a poor characterization.

Another example of the poor characterization of resonant wood comes from Fox Products Corporation – the experiment with the black walnut bassoon. A bassoon was built from black walnut (*Juglans nigra*) to see if another fine furniture wood could take the place of maple (*Acer* spp.). Both maple and black walnut have similar traditional and mechanical characteristics (Burns, *et al.* 1990). From the characters used to describe resonant wood now, the black walnut bassoon should have worked. When the instrument was tested in a concert hall, the sound did not project to the back of the room (Owen *pers.comm.*), a problem when the instrument in question was designed for a concert hall. Traditional characters did not eliminate black walnut from consideration. Since the characters typically used to select the resonant wood do not account for this problem, other characters need to be found.

Can the tapping method luthiers use help separate the resonant wood from the non-resonant? Since tapping directly tests for dampening of individual timbers, it seems to be a reasonable and easy method to separate the truly resonant timber of the resonant species used in bassoons. The purpose of this study was to determine if tapping could be used as a method of determining resonance. Samples of resonant and non-resonant wood for bassoon were tapped, the sounds recorded and measured using a sound program common in music circles, and the resulting data were analyzed using MANOVA and ANOVA. If successful, tapping can be used as an additional character for choosing resonant timber from among the known resonant species, reducing time and money spent and timber wasted during the manufacturing process.

Methods

To test whether tapping can differentiate resonant and non-resonant wood, samples from every species used to create bassoons were collected and shaped, along with a known non-resonant wood. Pen blanks were chosen because the wood quality needed to turn the wood on a microlathe, a miniature lathe used to create the body of homemade pens, is similar to that of resonant wood. Blanks of African blackwood (*Dalbergia melanoxylon*), hard maple (*Acer nigrum, A. saccharum*), mountain maple (*A. pseudoplatanus*), pear (*Pyrus communis*), soft maple (*A. rubrum, A. saccharinum*), and the non-resonant wood, black walnut (*Juglans nigra*), were obtained. They were then shaped to a uniform size (1.2 cm x 1.2 cm x 11 cm) using a vertical mill (Sharp Vertical Milling Machine) and a modified rabbiting bit. A total of 184 samples were

used (Appendix 1), representing timber of parallel grain (radial and tangential faces) and cross grain (grain generally $20^{\circ} - 45^{\circ}$ from horizontal axis).

A sclerometer was created to hold the pen blanks, and it was built with a special release for the hammer to keep the force of the blow constant for each sample. All samples were struck on each face (radial, tangential, 'cross-grain') in an 80% sound-proof room, and the tap was recorded with a recorder attached to an iPod, a portable recording/playing device (iPod®, Apple Corp.). The taps need to be easily distinguished without very sensitive recorders for this approach to be used in the field, and the iPod is a commonly owned appliance. In Adobe Soundbooth, a sound modification program (Adobe Systems Incorporated 2006-2009), 272 recordings were clipped to the same size and examined with Sonic Visualiser, a free sound analysis program from the University of London (Cannam 2005-2011).

Research over the years has indicated the beginning transients (including the lower partial frequencies) are important to an instrument's sound (Saldanha and Corso 1964). As a result, the lowest peak and melodic partials and their decibel levels were measured, along with the most powerful peak and melodic partials. A partial frequency is a simple frequency that comprises a complex sound. Decibel level is a measure of intensity, a ratio of power of one observation to a pre-determined reference. Each sound file was clipped to three seconds in length in Adobe Soundbooth and then uploaded to Sonic Visualser.

A spectrogram, an intensity plot of a sound that covers the entire length of the sound (Smith 2007), was created in Sonic Visualser to distinguish the partial frequencies of the complex sound created by the tap. Most studies of this kind use a spectrum instead; however, a spectrogram is more sensitive to differences among samples created from the same material (i.e. wood), whereas the spectrum is more useful for distinguishing samples of different materials (i.e. metal and wood). Program defaults were kept for the analysis (concert A = 440 Hz, y-axis interpolation = linear, x-axis interpolation = linear, window = Hann function). Peak and melodic frequency spectrograms of Channel 1 were created, along with a spectrum. The peak frequency spectrogram shows those partial frequencies that were stronger than the adjacent partials over the entire length of the sound, the melodic frequency spectrogram shows only those frequencies most commonly used in music over the entire length of the sound, and the spectrum shows the frequency analysis used to create the spectrograms at a specific point. From the spectrum, the

first three frequencies seen were recorded. The data were then examined in R, a free statistical package (R Development Core Team 2008).

The data were checked for any violations of statistical assumptions before being examined with MANOVA and ANOVA in R. Data were checked for outliers, normality, and independence. Outliers where revealed by plotting the ordered squared Mahalanobis distances (MD) of the data points against the empirical distribution function of MD^2. Normality was verified with a Q-Q plot. Independence of the variables was confirmed using paired scatterplots. The non-negative frequency data had to be transformed to achieve normality using the natural log. MANOVAs, an analysis technique that determines if two or more groups are statistically different using every variable measured in the study, were generated using wood type (resonant and non-resonant) and face (radial, tangential, 'cross-grain') as dependent factors. ANOVAs, an analysis technique that determines if two or more groups are statistically different using one variable measured in the study, were generated automatically using type as the dependent factor. Significance for all the tests was set at $\alpha = 0.05$.

Results

MANOVAs were generated from spectrum and spectrogram data and showed the partial frequencies within the tapped sounds produced could differentiate resonant and non-resonant wood. ANOVAs generated automatically after the MANOVAs showed many of the variables measured were also significantly different between the resonant and non-resonant species. Samples were separated by zone the species inhabit (temperate or tropical) and porosity (ring-porous or diffuse-porous). The architecture of temperate wood is much looser than the tropical species (i.e. more open space, lower density) and diffuse-porous woods have many more vessels with thinner walls, all of which could confound the results. Overall, neither of these issues changed the results. However, they did affect which variables were significant for differentiating resonant and non-resonant wood. Since orientation has an effect on dampening, the data were also examined by face ('cross-grain', radial, and tangential).

Resonant vs. Non-resonant Woods. MANOVA showed the resonant wood to be statistically different from the non-resonant *Juglans nigra* (black walnut) when all the faces were combined, when only the temperate species were considered, and when the ring-porous species were considered alone (Table 1). When data from all the faces were combined, the univariate ANOVA

showed the resonant wood to be statistically different from the non-resonant *J. nigra* using the following variables: hertz of the lowest melodic partial, hertz of the second spectrum block, and the hertz of the third spectrum block overall. Resonant wood was statistically different from the non-resonant *J. nigra* using the same variables when only the temperate species were examined and when only the ring-porous species were examined; the resonant wood was statistically different from the non-resonant *J. nigra* using decibel level of the lowest melodic partial and hertz of the lowest peak partial; the same results were found when only the temperate species were considered alone; the resonant wood was statistically different from the non-resonant *J. nigra* using hertz of the strongest peak partial and hertz of the strongest melodic partial when only the temperate species and the ring-porous species were examined respectively. The decibel level of the lowest peak partial separated the resonant wood from the non-resonant *J. nigra* when only the temperate species were examined; the decibel level of the strongest peak partial separated the resonant wood from the non-resonant *J. nigra* when only the ring-porous species were examined (Table 2).

The variables that separated the resonant woods from the non-resonant *Juglans nigra* (black walnut) were lower in general for *J. nigra* than in the resonant woods (Table 3). The decibel levels of lowest peak, lowest melodic, and strongest melodic partials were higher in the resonant woods, as well as the hertz of the strongest peak and melodic partials and third spectrum block. The hertz of the lowest peak and lowest melodic partials and the second spectrum block were higher in *J. nigra*.

The faces of the resonant wood could be separated statistically no matter the zone of growth. In the non-resonant *J. nigra*, the faces could also be separated statistically (Table 4). The decibel level of the strongest peak and lowest peak partials separated the faces no matter the zone of growth in the resonant woods, as well as the hertz of the strongest and lowest peak partials and the hertz of the third spectrum block; the decibel level of the strongest peak and lowest peak partials separated the faces, along with the hertz of the strongest and lowest peak partials and the hertz of the lowest melodic partial in the temperate resonant woods; the decibel levels of lowest melodic and peak partials separated the faces in the non-resonant *J. nigra* (Table 5).

'Cross-grain' Face. When only the 'cross-grained' samples were studied, MANOVA showed the resonant wood to not be statistically different from the non-resonant *Juglans nigra* (black walnut)

overall and when only the temperate species were considered; but the resonant wood was statistically different from the non-resonant *J. nigra* when the ring-porous species were considered alone (Table 1). The decibel levels and hertz of the strongest peak and melodic partials separated the resonant woods from the non-resonant *J. nigra* when only the ring-porous species were examined (Table 2).

The variables that separated the resonant woods from the non-resonant *Juglans nigra* (black walnut) were lower in general for *J. nigra* than in the resonant woods (Table 3). The decibel levels of the strongest peak, strongest and lowest melodic peak partials were higher in the resonant woods, as well as the hertz of the strongest peak and melodic partials.

Radial Face. When only the radial face samples were studied, MANOVA showed the resonant wood to be statistically different from the non-resonant *Juglans nigra* (black walnut) overall, when only the temperate species were considered, and when the ring-porous species were considered alone (Table 1). The univariate ANOVA showed the resonant wood to be statistically different from the non-resonant *J. nigra* using hertz of the lowest melodic partial, decibel level of the lowest melodic partial, hertz of the lowest peak partial, hertz of the second spectrum block, and the hertz of the third spectrum block overall. The same results were found when only the temperate species were examined, and when only the ring-porous species were examined. The decibel level of the strongest peak partial separated the resonant woods from the non-resonant *J. nigra* when only the temperate species were considered. Hertz of the strongest peak partial and the first spectrum block separated the resonant woods from the non-resonant *J. nigra* when only the ring-porous species were examined (Table 2).

The variables that separated the resonant woods from the non-resonant *Juglans nigra* (black walnut) were higher in general for *J. nigra* than in the resonant woods (Table 3). The decibel level of the strongest peak partial, hertz of the lowest peak and lowest melodic partials were higher in *J. nigra*, as well as the hertz of the second and third spectrum blocks. The decibel level of the lowest melodic partial, hertz of strongest peak partial, and the hertz of the first spectrum block were greater in the resonant woods.

Tangential Face. When only the tangential face samples were studied, MANOVA showed the resonant wood to be statistically different from the non-resonant *Juglans nigra* (black walnut) overall, when only the temperate species were considered, but not when the ring-porous species

were considered alone (Table 1). The univariate ANOVA showed the resonant wood to be statistically different from the non-resonant *J. nigra* using hertz of the lowest melodic partial, hertz of the second spectrum block, and the hertz of the third spectrum block overall. These results were also found when only the temperate species were examined, and when only the ring-porous species were examined. Decibel level of the lowest melodic partial and hertz of the lowest peak partials separated the resonant woods from the non-resonant *J. nigra* overall and when only the temperate species were considered. Hertz of the strongest peak partial separated the resonant woods from the non-resonant *J. nigra* when only the temperate species were considered and when only the ring-porous species were examined. Decibel level of the lowest peak partial and hertz of the strongest melodic partial separated the resonant woods from the non-resonant *J. nigra* when only the temperate species were considered (Table 2).

The variables that separated the resonant woods from the non-resonant *Juglans nigra* (black walnut) were higher in general for *J. nigra* than in the resonant woods (Table 3). The decibel level of the lowest melodic partial, hertz of the strongest and lowest peak partials, and the hertz of the second and third spectrum blocks were greater in *J. nigra*. Decibel levels in the lowest peak partial and hertz of the strongest and lowest melodic partial were greater in the resonant woods.

The faces were significantly different from each other within type (resonant, non-resonant) no matter the zone of origin (temperate, tropical) or porosity (ring-porous, diffuse-porous). Faces among the resonant woods were significantly different from each other overall and when considering the temperate (and consequently diffuse-porous) woods. Non-resonant wood (all temperate, all ring-porous species) faces were also significantly different from each other (Table 4). Frequencies of the strongest and lowest peak partials were the only significant variables among the resonant wood faces, and the non-resonant wood faces had no significant variables differentiating the faces (Table 5).

Overall, the frequency of the lowest melodic partial, along with the second and third frequencies from the spectrum, differentiated the resonant and non-resonant woods. This result was found in the overall and radial datasets. Decibel level of the lowest melodic partial and the frequency of the lowest peak partial were significant for the overall and temperate datasets. The frequency of the strongest peak partial was significant with the temperate and ring-porous datasets.

Discussion

The purpose of this study was to determine if tapping can be used to differentiate resonant wood from non-resonant wood. Weaker and lower partial frequencies combined to differentiate resonant wood from non-resonant wood, no matter the zone of origin (temperate or tropical) or the porosity of the wood (diffuse- or ring-porous). Significance was affected by the face (radial, tangential, 'cross-grain') studied – the radial and tangential faces were significantly different between resonant and non-resonant wood no matter the zone of origin or the porosity, whereas the 'cross-grain' face was significantly different between resonant and non-resonant wood only when the ring-porous species were examined alone. The intriguing information from the study turned out to be the significant variables and the information from the faces themselves.

The lower peak and melodic partial frequencies differentiated the resonant from the non-resonant wood in every longitudinal face, except when the 'cross-grain' was examined. These partials could be connected to the structure of the resonant and non-resonant wood and how each cell was cut. Both faces are dominated by longitudinally cut fibers and vessels, which are structural in nature and have a dense cellulose matrix. If these structural cells were alone, it would be natural to assume the partials distinguishing the wood types would be strong and high. However, the significant partials were low and weaker, indicating the important cells in these faces were the parenchyma. Parenchyma is cut horizontally in the tangential face, exposing each distinct cell wall layer, and vertically in the radial face, exposing only one layer. The layers in these softer cells also have a thin cell wall, because parenchyma does not play a structural role in wood. It is likely that parenchyma is lowering the intensity of the tapped sound by absorbing more energy than a fiber or vessel would, reducing the importance of a high, strong partial. The increased presence of axial parenchyma in *Juglans nigra* could also be playing a role in deadening the tap for the non-resonant wood. Axial parenchyma is cut vertically in all the longitudinal faces, exposing more lignin from these already soft cells and causing potential 'dead' zones that absorb more vibrational energy. This could help explain why an instrument made from *Juglans nigra* has poor sound projection – the cellular structure prevents sound travelling to the back of a concert hall by removing the energy needed to carry it.

The faces formed from the orientation and cut of the cells also react and act on vibration in different ways. Only the radial and tangential faces showed significant differences between

resonant and non-resonant wood. The non-result in the 'cross-grain' could be explained by the inability to control which angle between the radial and tangential faces the block was cut. It could be construed from this non-result that the angle of the 'cross-grain' matters, a result found by Schleske (1990) in regards to the violin. Violins and marimbas use the radial face preferentially for sound production, suggesting that this face is more resonant than the tangential face. The cut that exposes the radial face, the quartersawn cut, is also considered to be one of the most stable cuts. Even though both were good at distinguishing resonant and non-resonant wood, that could have been the result of the cellular organization in the species concerned and not the inherent resonance abilities of a specific face. If so, it would make sense that a 'cross-grain' face with a more radial orientation would be preferable to one with a more tangential orientation, again shown by Schleske's study on violin sound board arches. 'Cross-grain' faces with a more tangential orientation can be seen in wood with sharply curved growth rings using a vertical bore. This would account for the loss of resonance in some wood used for bassoon manufacture (Owen *pers. comm.*). The wood is resonant until the preferred 'cross-grain' faces are carved away. This study also showed that each face is significantly different from the others in resonant wood and not significantly different for *Juglans nigra.* The results indicate that face orientation matters for resonance.

Aside from Schleske, there have not been any tapping studies concerned with wood face resonance. However, that does not mean it is not a useful technique. It only suggests that this can be a new avenue for research. Hutchins and Fielding (1968) found that tapping can be useful for finding resonant and non-resonant spots within a timber. Although this is ultimately more useful for instruments that are carved and when the instrument maker can stop at just the right moment before resonant wood becomes non-resonant, it can still be used for instruments that require machining. The turning blanks can be tapped on each exposed face before the initial shaping and after the bore is drilled, eliminating all the dull sounding wood before it can be wasted, reducing work time. Identifying non-resonant spots within a resonant wood could also provide more information on the nature of wood resonance by direct comparison. Resonance is so complicated that a simple, inexpensive test such as tapping should not be ignored in favor of expensive gadgetry.

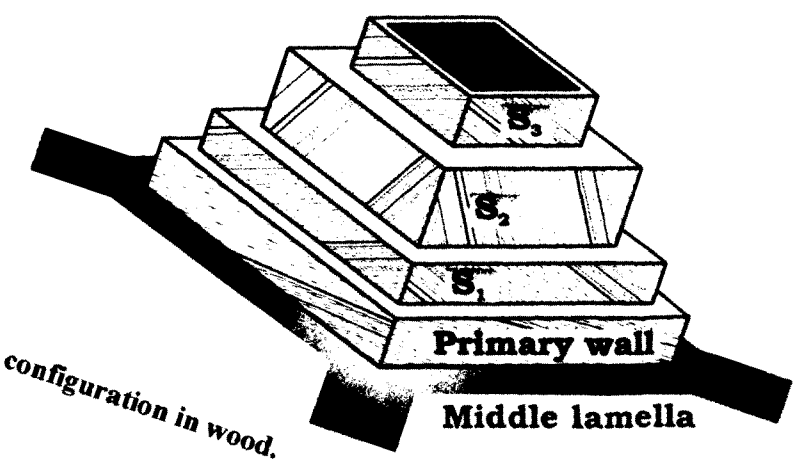

Figure 1 Cell wall configuration in wood.

Table 1 Results of MANOVA carried out on tapping data by type (resonant or non-resonant, df = 1) separated by face ('cross-grain', radial, tangential), location (temperate or tropical) and porosity (ring- or diffuse-porous). Significance set at $\alpha = 0.05$. Frequency data transformed with natural log. Numerator Df = 12 for all datasets. α = the boundary for statistical significance for observations in a dataset, N = number of observations, Df = degrees of freedom (N-1), den Df = denominator degrees of freedom, F (F-test statistic) = explained variance divided by unexplained variance, p (p-value) = the probability of getting an extreme test statistic from an observation, assuming the null hypothesis is true (Heath 1995).

Datasets	N	den Df	F	p
All Faces	264	252	7.65	<0.0001
temperate species	215	203	8.04	<0.0001
ring-porous species	133	121	4.44	<0.0001
'cross-grain'	101	89	1.014	0.4
temperate species	86	74	1.13	0.4
ring-porous species	50	38	3.0	0.005
radial	81	69	7.08	<0.0001
temperate species	64	52	6.63	<0.0001
ring-porous species	41	29	3.14	0.006
tangential	80	68	4.54	<0.0001
temperate species	63	51	5.84	<0.0001
ring-porous species	40	28	1.69	0.1

Table 2 Results of ANOVAs generated after MANOVA on tapping data by type (resonant or non-resonant, df = 1) separated by face ('cross-grain', radial, tangential), location (temperate or tropical) and porosity (ring- or diffuse-porous). Significance set at $\alpha = 0.05$. * indicates significant results with $p \leq 0.05$, † indicates nearly significant results $0.05 < p < 0.1$. Frequency data transformed with natural log. The number of observations (N) is divided into a. entire dataset, b. temperate species, c. ring-porous species. Numerator Df = 12 for all datasets. α = the boundary for statistical significance for observations in a dataset, N = number of observations, Df = degrees of freedom (N-1), den Df = denominator degrees of freedom, F (F-test statistic) = explained variance divided by unexplained variance (Heath 1995). dB = decibel level – a measure of intensity, Hz = hertz (frequency).

	All Faces (N = 264[a], 215[b], 133[c])		'Cross-Grain' (N = 101[a], 86[b], 50[c])		Radial (N = 81[a], 64[b], 41[c])		Tangential (N = 80[a], 63[b], 40[c])	
Variable	**den Df**	**F**	**den Df**	**F**	**den Df**	**F**	**den Df**	**F**
dB of strongest peak partial	252	0.28	89	0.72	69	2.65	68	0.69
temperate	203	0.0074	74	0.0092	52	7.1*	51	1.23
ring-porous	121	2.49	38	6.14*	29	0.16	28	0.065
dB of lowest peak partial	252	2.3	89	0.34	69	2.91†	68	1.86
temperate	203	5.74*	74	0.12	52	3.95†	51	5.78*
ring-porous	121	1.0	38	0.81	29	0.14	28	1.38
dB of strongest melodic partial	252	1.22	89	0.16	69	0.65	68	1.2
temperate	203	0.14	74	0.18	52	0.5	51	0.54
ring-porous	121	5.77*	38	4.98*	29	0.54	28	3.29†
dB of lowest melodic partial	252	21.05*	89	0.062	69	18.025*	68	14.36*
temperate	203	24.6*	74	0.71	52	15.83*	51	16.44*
ring-porous	121	2.3	38	1.74	29	4.85*	28	2.89†
# of strong partials	252	0.8	89	0.13	69	0.19	68	0.97
temperate	203	0.076	74	0.0067	52	0.015	51	0.85
ring-porous	121	3.57†	38	1.62	29	2.12	28	0.63
Hz of strongest peak partial	252	2.41	89	1.48	69	0.063	68	3.81†
temperate	203	8.33*	74	0.34	52	1.37	51	10.48*
ring-porous	121	14.6*	38	5.97*	29	8.3*	28	4.15*
Hz of lowest peak partial	252	22.79*	89	0.84	69	12.37*	68	19.09*
temperate	203	30*	74	0.91	52	12.83*	51	47.29*
ring-porous	121	2.29	38	0.17	29	5.29*	28	0.025
Hz of strongest melodic partial	252	0.56	89	2.28	69	1.44	68	3.026†
temperate	203	4.24*	74	0.33	52	3.84†	51	6.07*
ring-porous	121	7.63*	38	12.055*	29	0.79	28	0.039
Hz of lowest melodic partial	252	32.66*	89	0.76	69	26.043*	68	24.0*
temperate	203	35*	74	0.98	52	32.9*	51	24.37*
ring-porous	121	6.51*	38	0.015	29	4.29*	28	6.9*
Hz of 1st spectrum block	252	1.4	89	0.81	69	3.19†	68	0.0024
temperate	203	1.32	74	0.51	52	3.12†	51	0.08
ring-porous	121	1.036	38	0.73	29	4.62*	28	0.1
Hz of 2nd spectrum block	252	11.05*	89	0.073	69	8.68*	68	7.24*
temperate	203	10.42*	74	0.74	52	6.0*	51	0.023*
ring-porous	121	4.3*	38	1.062	29	6.33*	28	4.4*
Hz of 3rd spectrum block	252	16.53*	89	0.48	69	12.38*	68	8.54*
temperate	203	15.43*	74	1.61	52	9.1*	51	6.48*
ring-porous	121	5.63*	38	0.68	29	7.33*	28	4.33*

Table 3 Averages of variables used in study, separated by type (resonant, non-resonant). dB = decibel level – a measure of intensity, Hz = hertz (frequency).

	Non-resonant				Resonant			
Variable	**Overall**	**'Cross-grain'**	**Radial**	**Tangential**	**Overall**	**'Cross-grain'**	**Radial**	**Tangential**
dB of strongest peak partial	-18.87	-19	-18.6	-19.29	-18.79	-18.65	-19.18	-18.32
dB of lowest peak partial	-22.093	-21.78	-21.64	-21.13	-21.43	-22.35	-21.84	-20.72
dB of strongest melodic partial	-21.38	-21.24	-21.12	-21.17	-21.18	-21.21	-21.33	-21.23
dB of lowest melodic partial	-24.76	-23.70	-23.32	-22.29	-23.043	-25.29	-22.72	-23.07
# of strong partials	1	1	1	1	1	1	2	1
Hz of strongest peak partial	185.49	183.046	188.21	185.41	183.85	195.69	204.2	150.44
Hz of lowest peak partial	104.12	98.44	96.57	90.22	75.81	104.95	82.053	46.47
Hz of strongest melodic partial	159.25	146.07	164.0	126.47	159.78	182.63	163.14	151.56
Hz of lowest melodic partial	66.083	59.025	60.56	47.15	52.88	67.51	50.71	53.26
Hz of 1st spectrum block	24.58	25.33	21.91	35.79	27.04	23.64	27.90	26.82
Hz of 2nd spectrum block	151.13	142.0024	146.0	135.74	133.099	155.66	128.47	128.15
Hz of 3rd spectrum block	216.015	207.48	206.52	190.08	191.49	220.6	185.23	185.26

Table 4 Results of MANOVA carried out on tapping data by face (radial, tangential, 'cross-grain', df (degrees of freedom) = 2) separated by resonance (resonant, non-resonant). Significance set at $\alpha = 0.05$. Frequency data transformed with natural log. α = the boundary for statistical significance for observations in a dataset, N = number of observations, Df = degrees of freedom (N-1), num Df = numerator degrees of freedom, den Df = denominator degrees of freedom, F (F-test statistic) = explained variance divided by unexplained variance, p (p-value) = the probability of getting an extreme test statistic from an observation, assuming the null hypothesis is true (Heath 1995).

Datasets	N	num Df	den Df	F	p
All zones, resonant	358	24	334	5.014*	<0.0001
All zones, non-resonant	170	24	146	1.6*	0.05
Temperate, resonant	260	24	236	6.007*	<0.0001

Table 5 Results of ANOVAs generated after MANOVA on tapping data by by face ('cross-grain', radial, tangential). Significance set at α = 0.05. Frequency data transformed with natural log. α = the boundary for statistical significance for observations in a dataset, N = number of observations, Df = degrees of freedom (N-1), num Df = numerator degrees of freedom, den Df = denominator degrees of freedom, F (F-test statistic) = explained variance divided by unexplained variance, p (p-value) = the probability of getting an extreme test statistic from an observation, assuming the null hypothesis is true (Heath 1995). dB = decibel level – a measure of intensity, Hz = hertz (frequency).

	All zones, resonant N = 358 num df = 24, den df = 334		All zones, non-resonant N = 170 num df = 24, den df = 146		Temperate, resonant N = 260 num df = 24, den df = 236	
Variable	F	p	F	p	F	p
dB of strongest peak partial	7.032	0.001	1.2	0.31	8.72	0.00028
dB of lowest peak partial	3.14	0.046	0.76	0.47	6.68	0.0017
dB of strongest melodic partial	0.85	0.43	0.56	0.57	0.022	0.98
dB of lowest melodic partial	1.98	0.14	3.17	0.047	1.057	0.35
# of strong partials	0.61	0.54	0.3	0.74	0.82	0.44
Hz of strongest peak partial	17.91	< 0.0001	1.33	0.27	25.16	< 0.0001
Hz of lowest peak partial	43.35	< 0.0001	3.79	0.027	74.26	< 0.0001
Hz of strongest melodic partial	1.93	0.15	1.55	0.22	1.67	0.19
Hz of lowest melodic partial	2.8	0.064	3.93	0.023	3.88	0.023
Hz of 1st spectrum block	2.9	0.057	1.83	0.17	2.47	0.089
Hz of 2nd spectrum block	1.65	0.2	1.88	0.16	0.3	0.74
Hz of 3rd spectrum block	3.2	0.043	1.29	0.28	0.79	0.45

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Appendix 1 Origin of samples used in study.

Sample	Name	Location
CA001-CA010	black walnut (*Juglans nigra*)	USA: Iowa
JSH001-JSH002	black walnut (*Juglans nigra*)	Superior Hardwoods
JWC001-JWC028	black walnut (*Juglans nigra*)	USA: Pennsylvania
LL001-LL016	black walnut (*Juglans nigra*)	USA: Michigan
LTL001-LTL017	black walnut (*Juglans nigra*)	USA: Indiana
REE001-REE010	black walnut (*Juglans nigra*)	USA: Virginia
DLTL002. DLTL007	African blackwood (*Dalbergia melanoxylon*)	Logs to Lumber
DWC002-DWC025	African blackwood (*Dalbergia melanoxylon*)	Woodcraft.com
GW001-GW012	African blackwood (*Dalbergia melanoxylon*)	South Africa
A3Fox001-A3Fox007	hard maple (*Acer saccharum*)	USA: Pennsylvania
A3WC001-A3WC020	hard maple (*Acer saccharum*)	Woodcraft.com
LTLHM001-LTLHM009	hard maple (*Acer* spp.)	USA: Indiana
ACPSFox002-ACPSFox018	mountain maple (*Acer pseudoplatanus*)	Europe
LLP001-LLP005	pear (*Pyrus* spp.)	USA: Michigan
PWT001-PWT005	pear (*Pyrus* spp.)	Switzerland
A2002	soft maple (*Acer saccharinum*)	USA: Oklahoma
A2WC002	soft maple (*Acer saccharinum*)	Woodcraft.com
ACRUFox001-ACRUFox029	soft maple (*Acer rubrum*)	USA: Pennsylvania
ACRUOK001-ACRUOK006	soft maple (*Acer rubrum*)	USA: Oklahoma
LTLSM001-LTLSM015	soft maple (*Acer*spp.)	USA: Indiana

CHAPTER 5: THE POTENTIAL OF RED ALDER (*ALNUS RUBRA*) AND ALASKA PAPER BIRCH (*BETULA NEOALASKANA*) AS BASSOON RESONANT WOODS

Introduction

The Eastern Deciduous Forest is under attack by pestilence and disease. American elm (*Ulmus americana*) populations were decimated by Dutch elm disease during the 20th century (Karnosky 1979). American chestnut (*Castanea americana*) populations were destroyed by blight at the same time (Anagnostakis 1987). More recently, ash (*Fraxinus* spp.) have been attacked by the emerald ash borer, and maple (*Acer* spp.) and other common hardwoods have been damaged by the Asian hornedbeetle (Gandhi and Herms 2010). These attacks are changing the face of the Eastern Deciduous Forest, and the availability of quality timber is decreasing.

Bassoon manufacturers have exclusively used maple (*Acer* spp.) for the last 300 years to create classical and German system bassoons (Langwill 1971). European mountain maple (*Acer pseudoplatanus*) was the primary maple used until the 17th century when Karl Almenraeder started using the North American maples (*A. nigrum*, *A. rubrum*, *A. saccharinum*, *A. saccharum*) (Zadro 1975). The dangers of using maple exclusively are based on the facts that the eastern forest composition will be altered because of climate change (Dukes, *et al.* 2009) and sugar maple forests in the northeast are steadily declining from environmental factors and disease (Horsley, *et al.* 2002). Only a few attempts at using different woods were made because of the expense and time needed to create a bassoon.

One of these attempts was made at Fox Products Corporation. They tried a temperate wood with similar features to maple, black walnut (*Juglans nigra*), but the trial was unsuccessful (Owen, *pers. comm.*). Black walnut has similar aesthetic and mechanical features to maple. The wood is straight, defect-free, fine-grained, and the tree grows large enough to produce turning blocks with straight growth rings. Its specific gravity is similar to maple (United States Forest Products Laboratory 1974) and is commonly used as a turning wood. It seemed logical for Fox to create a black walnut bassoon. It projected its sound well in the workroom, but the sound did not reach the back when played in a concert hall (Owen, *pers. comm.*). This test was important, because it showed not all temperate hardwoods are created equal in regards to bassoon resonance. The test also showed that material plays a role in an instrument's sound.

Some studies on alternative materials have been done for other instruments. Brancheriau *et al.* (2006) looked at different tropical hardwoods for the marimba. They found some woods work better than others. Yoshikawa *et al.* (2008) examined two woods used to make a biwa (a traditional Japanese stringed instrument) soundboards. They also found some woods work better than others. Alternative woods for the bassoon might lie in a heavily forested area that is isolated from the troubles that plague the Eastern Deciduous Forest.

Alaska has 10 million acres of angiosperm (hardwood) species and 63 million acres of gymnosperms (softwoods) (Smith, *et al.* 2001). Viereck and Little (2007) reported 12 tree species, six of which were hardwoods (Table 1). Two of the hardwood species, red alder *(Alnus rubra)* and Alaska paper birch (*Betula neoalaskana*), have specific gravities similar to maple and the violin resonant wood *Picea abies*. Red alder and Alaska paper birch are also the only species to reach the minimum girth needed to produce turning blanks with straight growth rings (United States Forest Products Laboratory 1974).

Will Alaska paper birch or red alder work for bassoon manufacture? Neither species are known for being furniture woods capable of being turned thinly (Burns, *et al.* 1990); however, both are diffuse-porous temperate woods with similar habitats to maple. I hypothesize that both species should work, based on their similarity to maple. The two Alaska species were compared to both the temperate resonant woods and black walnut by cluster analyses and MANOVA, using anatomical characters determined to be significant in a previous study and specific gravity (relative density).

Methods

In order to determine whether the Alaska hardwoods could be classified as resonant, red alder *(Alnus rubra)* and Alaska paper birch (*Betula neoalaskana*) were compared to known temperate resonant species (*Acer* spp. and *Pyrus communis*) and a known non-resonant species (*Juglans nigra*) using anatomy, approximate specific gravity, and tapping. *Alnus rubra* was harvested as a log with a 12 inch (30.48 cm) diameter on Prince of Wales Island, split into quarters, the ends sealed with paraffin wax, and seasoned for over a year. *Betula neoalaskana* was harvested as a log with an 11 inch (27.94 cm) diameter in the Tanana Valley region of interior Alaska and seasoned in a warm, humid room for over a year. Samples of *Acer*, *Pyrus communis*, and *Juglans nigra* were bought as pen blanks. Pen blanks were chosen because the quality of wood used for

making pens is similar to the quality of resonance wood. In total, two trees were used to represent *A. rubra* for the tapping portion of the study, one tree was used to represent *B. neoalaskana*, eight trees were used to represent the resonant woods, and six trees were used to represent the non-resonant *Juglans nigra*; two trees were used to represent *A. rubra* for the anatomy portion of the study, 10 trees were used to represent *B. neoalaskana*, seven trees were used to represent the resonant woods, and five woods were used to represent *J. nigra*. The woods were all subjected to anatomical analysis, a tapping test, and their specific gravity approximated, followed by a statistical comparison.

Anatomy. Slides containing each face were created by sectioning each sample face (transverse, tangential, radial, 'cross-grain') to 10 μm using an AO 860 sliding microtome. The sections were then permanently mounted with Permount (Permount™, Fisher Scientific) unstained. Slides containing loose cells were created by macerating a portion of the sample with a 1:4:5 solution of hydrogen peroxide: distilled water: glacial acetic acid. The macerated tissue was then dried to an adhesive coated slide, stained for five minutes with a 0.01% solution of safranin (Safranin O, Fisher Scientific), and permanently mounted with Permount (Permount™, Fisher Scientific). In total, 40 section slides and 40 maceration slides were created and imaged in this study (Appendix 1).

To collect dimensional and cell wall variable data, the slides were imaged with a compound light microscope (LM) and a confocal laser scanning microscope (CLSM). Both stained and unstained section slides were imaged with polarized and non-polarized light at 40X using a Nikon Alphaphot YS LM for measuring cell dimensions and the amount of crystalline structure (i.e. crystalline cellulose, crystals in axial parenchyma) exposed by face. To measure the cell dimensions obscured by sectioning, the maceration slides were imaged at 4X using an AmScope LM.

All the data were collected using Image J, a free measurement software provided by the National Institute of Health (Rasband 1997-2011). The variables measured were chosen based on a previous study comparing resonant and non-resonant bassoon woods. In that study the dataset was broken up into two subsets – temperate species only and ring-porous species only. The dataset was split because the tropical species and the diffuse-porous species in the study could potentially skew the data. Tropical woods have a heavier concentration of cells within a

prescribed area, with thick cell walls; diffuse-porous species have a greater number of vessels between growth rings.

Specific Gravity. Specific gravity was approximated for each sample using a method in *Identifying Wood* by B. Hoadley (1990). Each sample was encased in a thin layer of paraffin wax to prevent water from penetrating the wood, which would change the weight of the wood. The sample was then inserted into a 100mL graduated cylinder that contained 50 mL water. The length of the submerged portion of the sample was measured, and that measurement was divided by the overall length of the sample. The resulting fraction was found on the x-axis of the regression graph provided by the book. The specific gravity was found by determining the intercept point of the fraction and the 15% moisture content regression line and locating the specific gravity on the y-axis that was parallel to the intercept.

Tapping. The logs collected were cut down to pen blanks (1.9 cm x 1.9 cm x 15.2 cm) and then shaped to a uniform size (1.2 cm x 1.2 cm x 11 cm) using a vertical mill (Sharp Vertical Milling Machine) and a modified rabbiting bit. A total of 40 samples were used (Appendix 2), representing timber of parallel grain (radial and tangential faces) and cross grain (grain 45° from horizontal axis).

A sclerometer – an instrument used to measure hardness (Terichow, *et al.* 1967) – was created to hold the pen blanks with a special release for the hammer in order to keep the force of the blow constant for each sample (Figure 1). In an 80% sound-proof room, the wood sample was placed in the clamp with the face being studied in front of the hammer. A recorder attached to an iPod (iPod®, Apple Corp.) was turned on to start the recording. The iPod (iPod®, Apple Corp.) was chosen because the taps need to be easily distinguished without very sensitive recorders for this approach to be used in the field. The hammer was released. After the hammer struck the sample, the hammer was caught automatically by the release mechanism. The recording was stopped. This was repeated with every sample on each of the faces being studied. In Adobe Soundbooth (Adobe Systems Incorporated 2006-2009) 40 recordings were clipped to the same size and examined with Sonic Visualiser (Cannam 2005-2011).

A spectrogram was created in Sonic Visualser to distinguish the partial frequencies of the complex sound created by the tap. Most studies of this kind use a spectrum instead; however, a spectrogram is more sensitive to differences among samples created from the same material (i.e.

wood), whereas the spectrum is more useful for distinguishing samples of different materials (i.e. metal and wood). Program defaults were kept for the analysis (concert A = 440 Hz, y-axis interpolation = linear, x-axis interpolation = linear, window = Hann function). Peak and melodic frequency spectrograms of Channel 1 were created, along with a spectrum. The peak frequency spectrogram shows those partial frequencies that were stronger than the adjacent partials over the entire length of the sound, the melodic frequency spectrogram shows only those frequencies most commonly used in music over the entire length of the sound, and the spectrum shows the frequency analysis used to create the spectrograms at a specific point. The variables used were determined from a previous study comparing resonant and non-resonant wood.

Analyses. The data were analyzed in R, a free statistical software package (R Development Core Team 2008). Datasets were all checked to see if they conformed to the assumptions needed to perform a valid statistical analysis – no outliers (data points that lie far away from the rest of the data in a graph (Whitlock and Schluter 2009)), the data is normal, and the variables are independent from each other. Outliers where revealed by plotting the ordered squared Mahalanobis distances (MD) of the data points against the empirical distribution function of MD^2. Normality was verified with a Q-Q plot. Independence of the variables was confirmed using paired scatterplots – graphs of data points plotted on a Cartesian plane using one variable as the x-axis and another as the y-axis (Whitlock and Schluter 2009). Clustering analyses, k-means clustering and principal components analysis (PCA), were used to determine within which group (resonant, non-resonant) red alder (*Alnus rubra*) and Alaska paper birch (*Betula neoalaskana*) fell. K-means clustering is a multivariate technique that attempts to partition observations into a predetermined number of groups, called k (Manly 2005). PCA takes the variables in the analysis and reorders them into uncorrelated components that maximize variability and uses those new components to group observations together based on similarity (Manly 2005). Multivariate analysis of variance (MANOVA), a way to determine if two or more groups are statistically different using more than one variable, was also used to determine if birch and alder are statistically different from the resonant species (*Acer* spp. and *Pyrus communis*).

Results

Clustering and MANOVA were used to determine if the Alaska woods (*Alnus rubra, Betula neoalaskana*) were more similar to the temperate resonant woods (*Acer* spp., *Pyrus communis*) or

a non-resonant temperate wood (*Juglans nigra*). The data were split up into two sets – anatomy/specific gravity and tapping. Overall, the Alaska woods were statistically different from the temperate resonant woods and clustered with *J. nigra* using the anatomical characters and specific gravity.

Anatomy. Cluster plots show alder (*Alnus rubra*) and birch (*Betula neoalaskana*) grouped together with *Juglans nigra* using the first two components (Figs. 2, 3), which encompassed 80.8% of the variability from the alder dataset and 70.8% of the variability from the birch dataset (Table 2). On average (Table 3), maximum vessel width *in situ*, axial parenchyma width, fiber length, vessel length *macerated*, and vessel width *macerated* were higher in the non-resonant group (black walnut, alder or birch). Number of vessels and estimated specific gravity were lower in the non-resonant group (black walnut, alder or birch). Both birch and alder were statistically different from the temperate resonant woods, as seen from the MANOVAs (Table 4). All variables separated the birch and alder from the resonant group except vessel width *macerated* in the dataset containing alder, as well as the dataset containing birch (Table 5).

Tapping. Cluster plots did not show any differentiation between the resonant and non-resonant wood, nor did it cluster the alder or birch with one wood type. MANOVA showed that alder and birch were statistically different from the resonant woods for all faces (Table 6). Univariate ANOVA showed the decibel levels of the lowest peak and melodic frequencies to be statistically different between the Alaska and resonant woods no matter the face; the lowest peak frequency was statistically different between the Alaska and resonant woods in the cross face; the strongest peak frequency was statistically different between alder and the resonant woods in the cross face and between birch and the resonant woods in the radial face; and the strongest melodic frequency was statistically different between birch and the resonant woods in the radial face (Table 7). On average, the decibel levels of the lowest peak and melodic partials were lower in the resonant woods compared to both alder and birch; the lowest peak frequency was higher in the resonant woods than in alder and birch. Both the strongest peak and strongest melodic partials were lower in the resonant woods compared to the Alaska woods (Table 8).

Discussion

The purpose of this study was to determine if the Alaska hardwoods, red alder *(Alnus rubra)* and Alaska paper birch (*Betula neoalaskana*), were more similar to the temperate resonant woods or

the non-resonant *Juglans nigra* (black walnut). Both the Alaska hardwoods clustered with *J. nigra* using anatomical characters and specific gravity, and MANOVA supported this. No clustering occurred using the tapping data; however, MANOVA showed the Alaska hardwoods were statistically different from the temperate resonant hardwoods. In this study, the Alaska hardwoods examined are non-resonant.

The cell types of the temperate resonant woods are smaller than those in the Alaska hardwoods and *J. nigra*. Larger diameter vessels created larger voids in wood, even when the resonant woods have more, smaller vessels per mm^2. These voids represent a wider space through which a vibration has to travel across another medium (e.g. air instead of the solid state of the cell wall). A change in medium necessitates a change in energy a vibration needs to move from one point to another. Large axial parenchyma exposes softer cell area to an instrument's vibration. Parenchyma cells have thicker primary walls and thinner secondary walls than the vessels and fibers (Chafe 1974), and which means there is less of the cellulose that does not absorb energy in parenchyma than in other cells and more lignin, which absorbs energy from vibration rather than reflecting it back. Axial parenchyma is also cut along its long axis in the faces exposed to an instrument's vibration, which also exposes more lignin than cellulose, causing a sink for vibrational energy. The other two cell types cut along their long axes are fibers and vessels, and a longer cell exposes more of that cut to an instrument's vibrational energy, causing another sink for energy. The larger cells of red alder *(Alnus rubra)* and Alaska paper birch (*Betula neolaskana*) would absorb energy from an instrument's vibration and cause dampening of the resulting sound.

In context to other studies, the results are similar but may not mean as much since the instruments studies were not woodwinds. Brancheriau *et al.* (2006) found that certain woods are better for making marimba bars than others, even though they are all tropical and share a similar habitat and mechanical properties. The less suitable woods had greater amounts of axial parenchyma, which is similar to this study. Yoshikawa *et al.* (2008) compared two biwa soundboards made with the traditional mulberry (*Morus alba*) and the non-conventional camphor (*Cinnamomum camphora*) and found the camphor to be an inferior choice. They did not examine the anatomical qualities; however, this study illustrates that not all hardwoods are created equal in context to resonance, even if they have similar mechanical and aesthetic qualities. Camphor has a specific gravity of 0.43 and mulberry 0.52, both similar to maple's range of 0.44 – 0.68 (United States Forest

Products Laboratory 1974). Unlike this study, neither species appears to have a large amount of axial parenchyma; however, camphor does have a large amount of oil cells, which could affect the resonance of the wood.

This study shows that not all temperate woods are created equal when it comes to bassoon manufacture. Maple is the ideal wood for bassoons, but using only a few species exclusively is dangerous. With the steady incursion of disease and invasive species, the vitality of the genus and its ability to compete in a dynamic system could be compromised to a point where high quality wood is no longer produced. The woods used in this study may not work; however, there are several species in North America that still have potential, and if these potential woods can be successfully used in bassoon manufacture, the industry could be protected from resource loss and the demands on maple could be lessened. Because the characters used in this study are not traditionally measured when describing wood, future work would include examining hardwood species throughout the United States that have a minimum girth of 1 meter and compiling a list of possible replacements.

Figure 1 Diagram of sclerometer used in study.

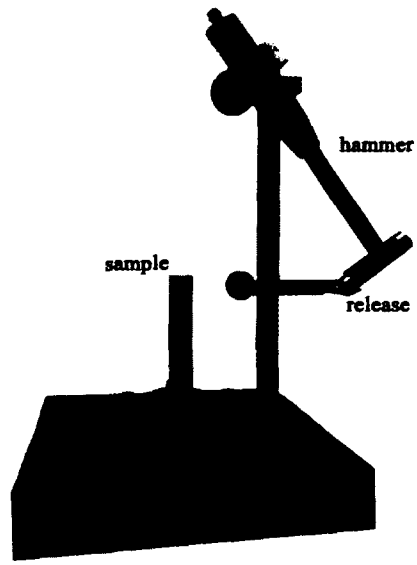

Figure 2 K-means cluster plot of known resonant woods (*Acer* spp. and *Pyrus communis*), red alder *(Alnus rubra),* and the non-resonant black walnut (*Juglans nigra*).

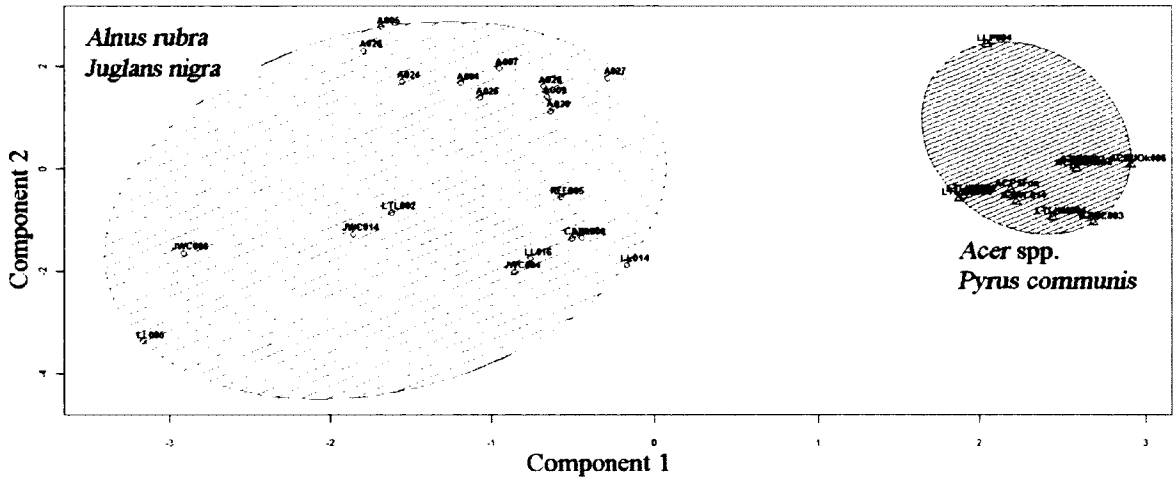

Figure 3 K-means cluster plot of known resonant woods (*Acer* spp. and *Pyrus communis*), Alaska paper birch (*Betula neoalaskana*), and the non-resonant black walnut (*Juglans nigra).*

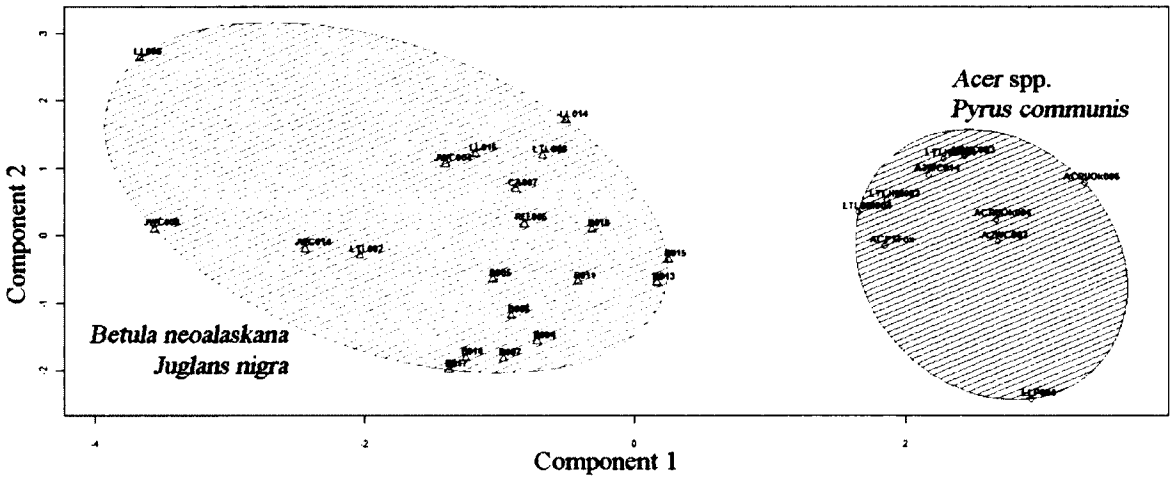

Table 1 Tree species found in Alaska that have a girth (trunk diameter) of at least 1 meter.

Common name	Scientific name
red alder	*Alnus rubra*
Alaska paper birch	*Betula neoalaskana*
Alaska cedar	*Chamaecyparis nootkatensis*
tamarack	*Larix laricina*
white spruce	*Picea glauca*
black spruce	*Picea mariana*
Sitka spruce	*Picea sitchensis*
balsam poplar	*Populus balsamifera*
quaking aspen	*Populus tremuloides*
black cottonwood	*Populus trichocarpa*
western hemlock	*Tsuga heterophylla*
mountain hemlock	*Tsuga mertensiana*

Table 2 Principal components of red alder and Alaska paper birch analyses.

Principal components	Standard Deviation		% Proportion of Variance		% Cumulative Proportion	
	Alder	Birch	Alder	Birch	Alder	Birch
PC1	1.79	1.9	45.75	51.36	45.75	51.36
PC2	1.57	1.17	35.02	19.46	80.77	70.82
PC3	0.77	0.81	8.457	9.389	89.23	80.207
PC4	0.54	0.79	4.112	9.017	93.342	89.224
PC5	0.46	0.68	3.048	6.566	96.39	95.791
PC6	0.42	0.45	2.552	2.936	98.941	98.726
PC7	0.27	0.3	1.059	1.274	100.0	100.0

Table 3 Variable averages of groups created from K-means analyses.

Variable	resonant woods	birch	alder	black walnut
number of vessels	13	6	16	4
maximum vessel width *in situ* (μm)	34.8	67.9	52.4	93.2
axial parenchyma width (μm)	1.3	12.6	13.9	15.2
fiber length (μm)	599.4	950.7	1103.3	1108.6
vessel length *macerated* (μm)	312.9	608.05	686.9	457.8
vessel width *macerated* (μm)	72.6	85.9	88.8	184.3
estimated specific gravity	0.6	0.6	0.4	0.6

Table 4 MANOVAs of analyses. Significance set at $\alpha = 0.05$. α = the boundary for statistical significance for observations in a dataset, N = number of observations, Df = degrees of freedom (N-1), den Df = denominator degrees of freedom, F (F-test statistic) = explained variance divided by unexplained variance, p (p-value) = the probability of getting an extreme test statistic from an observation, assuming the null hypothesis is true (Heath 1995).

Factors	N	num Df	den Df	F	p
alder, resonant woods	20	7	11	42.7	<0.001
birch, resonant woods	20	7	12	54.2	<0.001

Table 5 ANOVAs of variables used in analyses. Significance set at $\alpha = 0.05$. α = the boundary for statistical significance for observations in a dataset, N = number of observations, Df = degrees of freedom (N-1), den Df = denominator degrees of freedom, F (F-test statistic) = explained variance divided by unexplained variance (Heath 1995).

	Alder, resonant woods N=20, num Df=7, den Df=11		Birch, resonant woods N=20, num Df=7, den Df=12	
variable	F	p	F	p
number of vessels	0.39	0.5	5.2	0.04
maximum vessel width *in situ* (μm)	14.6	0.001	38.08	<0.0001
axial parenchyma width (μm)	96.7	<0.0001	86.8	<0.0001
fiber length (μm)	109.8	<0.0001	99.8	<0.0001
vessel length *macerated* (μm)	74.8	<0.0001	62.9	<0.0001
vessel width *macerated* (μm)	4.1	0.06	3.2	0.09
estimated specific gravity	161.3	<0.0001	6.8	0.02

Table 6 MANOVAs of tapping analyses. Significance set at α = 0.05. α = the boundary for statistical significance for observations in a dataset, N = number of observations, Df = degrees of freedom (N-1), den Df = denominator degrees of freedom, F (F-test statistic) = explained variance divided by unexplained variance, p (p-value) = the probability of getting an extreme test statistic from an observation, assuming the null hypothesis is true (Heath 1995).

	Alnus rubra vs. resonant woods N=12, Num Df=8, Den Df=3		*Betula neoalaskana* vs. resonant woods N=12, Num Df=8, Den Df=3	
Face	F	p	F	p
cross	170.9	0.0007	60.026	0.003
radial	50.9	0.004	100.62	0.001
tangential	22.194	0.0003	69.029	0.003

Table 7 ANOVAs of variables used in sound analyses. Significance set at α = 0.05. α = the boundary for statistical significance for observations in a dataset, N = number of observations, Df = degrees of freedom (N-1), den Df = denominator degrees of freedom, F (F-test statistic) = explained variance divided by unexplained variance (Heath 1995). dB = decibel level – a measure of intensity, Hz = hertz (frequency).

	cross				radial				tangential			
	alder		birch		alder		birch		alder		birch	
Variable	F	p	F	p	F	p	F	p	F	p	F	p
Strongest peak Hz	11.03	0.008	0.4	0.5	3.0	0.1	6.5	0.03	0.03	0.6	1.0	0.3
Lowest peak Hz	4.9	0.05	8.4	0.02	4.7	0.06	4.7	0.06	0.02	0.9	0.02	0.9
Lowest peak dB	744.2	<0.0001	600.6	<0.0001	222.6	<0.0001	259.3	<0.0001	40.02	<0.0001	51.3	<0.0001
Strongest melodic Hz	0.006	0.9	0.06	0.8	0.3	0.6	7.6	0.02	2.09	0.2	0.5	0.5
Lowest melodic Hz	0.6	0.5	0.04	0.9	0.2	0.7	0.08	0.8	0.6	0.4	1.05	0.3
Lowest melodic dB	131.6	<0.0001	129.6	<0.0001	182.06	<0.0001	163.8	<0.0001	48.8	<0.0001	53.2	<0.0001
2nd spectrum Hz	0.6	0.5	1.4	0.3	0.8	0.4	8.1	0.02	0.05	0.8	0.6	0.5
3rd spectrum Hz	0.9	0.4	2.8	0.1	1.7	0.2	2.3	0.2	0.9	0.4	0.5	0.5

Table 8 Averages of sound variables used in study. dB = decibel level – a measure of intensity, Hz = hertz (frequency).

Variable	*Alnus rubra*			*Betula neoalaskana*			resonant woods		
	cross	radial	tangential	cross	radial	tangential	cross	radial	tangential
Strongest peak Hz	231.0	201.0	169.8	189.4	230.8	243.6	169.006	140.3	202.8
Lowest peak Hz	59.4	51.0	51.1	50.9	50.8	50.7	94.9	90.2	53.4
Lowest peak dB	-30.8	-30.7	-30.2	-31.0	-31.0	-31.3	-20.7	-20.0	-21.7
Strongest melodic Hz	117.8	136.9	72.7	127.8	215.5	174.2	113.9	105.9	140.8
Lowest melodic Hz	50.3	49.1	49.5	48.3	49.0009	49.1	48.7	48.6	51.0
Lowest melodic dB	-32.8	-32.3	-31.7	-33.2	-33.0	-32.2	-22.5	-21.8	-23.3
2^{nd} spectrum Hz	117.9	150.2	113.5	102.0	98.6	106.0	138.4	131.02	117.4
3^{rd} spectrum Hz	179.2	209.4	338.5	148.7	156.3	163.6	202.6	183.5	176.1

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Appendix 1 Location information of samples used in anatomical study.

Specimen O.T.U.	Name	Location
CA007	black walnut (*Juglans nigra*)	USA: Iowa
JWC004	black walnut (*Juglans nigra*)	USA: Pennsylvania
JWC008	black walnut (*Juglans nigra*)	USA: Pennsylvania
JWC014	black walnut (*Juglans nigra*)	USA: Pennsylvania
LL005	black walnut (*Juglans nigra*)	USA: Michigan
LL014	black walnut (*Juglans nigra*)	USA: Michigan
LL016	black walnut (*Juglans nigra*)	USA: Michigan
LTL002	black walnut (*Juglans nigra*)	USA: Indiana
LTL008	black walnut (*Juglans nigra*)	USA: Indiana
REE005	black walnut (*Juglans nigra*)	USA: Virginia
A3WC003	hard maple (*Acer saccharum*)	USA: Eastern Deciduous Forest
LTLHM002	hard maple (*Acer* spp.)	USA: Indiana
LTLHM004	hard maple (*Acer* spp.)	USA: Indiana
ACPSFox	sycamore maple (*Acer pseudoplatanus*)	Europe
LLP004	pear (*Pyrus* spp.)	USA: Michigan
A2WC002	soft maple (*Acer saccharinum*)	USA: Eastern Deciduous Forest
ACRUOk006	soft maple (*Acer rubrum*)	USA: Oklahoma
ACRUOk004	soft maple (*Acer rubrum*)	USA: Oklahoma
A3WC014	hard maple (*Acer saccharum*)	USA: Eastern Deciduous Forest
LTLSM004	soft maple (*Acer* spp.)	USA: Indiana
A004	red alder (*Alnus rubra*)	USA: Alaska - Prince of Wales Island
A005	red alder (*Alnus rubra*)	USA: Alaska - Prince of Wales Island
A007	red alder (*Alnus rubra*)	USA: Alaska - Prince of Wales Island
A009	red alder (*Alnus rubra*)	USA: Alaska - Prince of Wales Island
A020	red alder (*Alnus rubra*)	USA: Alaska - Prince of Wales Island
A024	red alder (*Alnus rubra*)	USA: Oregon
A025	red alder (*Alnus rubra*)	USA: Oregon
A026	red alder (*Alnus rubra*)	USA: Oregon
A027	red alder (*Alnus rubra*)	USA: Oregon
A028	red alder (*Alnus rubra*)	USA: Oregon

Appendix 1 continued

Specimen O.T.U.	Name	Location
B004	Alaska birch (*Betula neoalaskana*)	USA: Alaska – Fairbanks
B005	Alaska birch (*Betula neoalaskana*)	USA: Alaska – Palmer
B006	Alaska birch (*Betula neoalaskana*)	USA: Alaska - Fort Richardson
B007	Alaska birch (*Betula neoalaskana*)	USA: Alaska –Tok
B011	Alaska birch (*Betula neoalaskana*)	USA: Alaska - Quartz Lake
B013	Alaska birch (*Betula neoalaskana*)	USA: Alaska - Parks Highway
B014	Alaska birch (*Betula neoalaskana*)	USA: Alaska - Richardson Highway
B015	Alaska birch (*Betula neoalaskana*)	USA: Alaska - King Mountain
B017	Alaska birch (*Betula neoalaskana*)	USA: Alaska - Elmendorf Airforce Base
B018	Alaska birch (*Betula neoalaskana*)	USA: Alaska - Montana Creek

Appendix 2 Location information of samples used in tapping study.

Specimen O.T.U.	Name	Face	location
B003	Alaska birch (*Betula neoalaskana*)	cross-grain'	USA: Alaska – Fairbanks
B007	Alaska birch (*Betula neoalaskana*)	cross-grain'	USA: Alaska – Fairbanks
B011	Alaska birch (*Betula neoalaskana*)	cross-grain'	USA: Alaska – Fairbanks
B009	Alaska birch (*Betula neoalaskana*)	cross-grain'	USA: Alaska – Fairbanks
B008	Alaska birch (*Betula neoalaskana*)	cross-grain'	USA: Alaska – Fairbanks
B010	Alaska birch (*Betula neoalaskana*)	cross-grain'	USA: Alaska – Fairbanks
B018	Alaska birch (*Betula neoalaskana*)	tangential, radial	USA: Alaska-Fairbanks
B016	Alaska birch (*Betula neoalaskana*)	tangential, radial	USA: Alaska-Fairbanks
B013	Alaska birch (*Betula neoalaskana*)	tangential, radial	USA: Alaska-Fairbanks
B017	Alaska birch (*Betula neoalaskana*)	tangential, radial	USA: Alaska-Fairbanks
B006	Alaska birch (*Betula neoalaskana*)	tangential, radial	USA: Alaska-Fairbanks
B014	Alaska birch (*Betula neoalaskana*)	tangential, radial	USA: Alaska-Fairbanks
CA009	black walnut (*Juglans nigra*)	cross-grain'	USA: Iowa
LTL-II	black walnut (*Juglans nigra*)	cross-grain'	USA: Indiana
JWC-VIII	black walnut (*Juglans nigra*)	cross-grain'	USA: Pennsylvania
JSH-II	black walnut (*Juglans nigra*)	cross-grain'	USA: Eastern Deciduous Forest
JWC-X	black walnut (*Juglans nigra*)	cross-grain'	USA: Pennsylvania
LTL-VIII	black walnut (*Juglans nigra*)	cross-grain'	USA: Indiana
REE-VIII	black walnut (*Juglans nigra*)	tangential, radial	USA: Virginia
JWC-II	black walnut (*Juglans nigra*)	tangential, radial	USA: Pennsylvania
JWC-IV	black walnut (*Juglans nigra*)	tangential, radial	USA: Pennsylvania

Appendix 2 continued

Specimen O.T.U.	Name	Face	location
LL007	black walnut (*Juglans nigra*)	tangential, radial	USA: Michigan
JWC-VI	black walnut (*Juglans nigra*)	tangential, radial	USA: Pennsylvania
JWC-XV	black walnut (*Juglans nigra*)	tangential, radial	USA: Pennsylvania
ACPSFox-III	European mountain maple (*Acer pseudoplatanus*)	cross-grain'	Europe
ACPSFox-VI	European mountain maple (*Acer pseudoplatanus*)	tangential, radial	Europe
A3WC-III	hard maple (*Acer saccharum*)	cross-grain'	USA: Eastern Deciduous Forest
A3WC-XIII	hard maple (*Acer saccharum*)	cross-grain'	USA: Eastern Deciduous Forest
A3WC-XIV	hard maple (*Acer saccharum*)	cross-grain'	USA: Eastern Deciduous Forest
A3Fox-I	hard maple (*Acer saccharum*)	tangential, radial	USA: Pennsylvania
PWT-III	pear (*Pyrus communis*)	cross-grain'	Switzerland
PWT-II	pear (*Pyrus communis*)	tangential, radial	Switzerland
LLP005	pear (*Pyrus* spp.)	cross-grain'	USA: Michigan
A010	red alder (*Alnus rubra*)	cross-grain'	USA: Oregon
A004	red alder (*Alnus rubra*)	cross-grain'	USA: Oregon
A007	red alder (*Alnus rubra*)	cross-grain'	USA: Alaska - Prince of Wales Island
A018	red alder (*Alnus rubra*)	cross-grain'	USA: Alaska - Prince of Wales Island
A022	red alder (*Alnus rubra*)	cross-grain'	USA: Alaska - Prince of Wales Island
A008	red alder (*Alnus rubra*)	tangential, radial	USA: Alaska-Prince of Wales Island
A017	red alder (*Alnus rubra*)	tangential, radial	USA: Oregon
A016-1	red alder (*Alnus rubra*)	tangential, radial	USA: Oregon
A016-2	red alder (*Alnus rubra*)	tangential, radial	USA: Oregon
A008-2	red alder (*Alnus rubra*)	tangential, radial	USA: Alaska-Prince of Wales Island
ACRUOk-II	soft maple (*Acer rubrum*)	tangential, radial	USA: Oklahoma
ACRUFox-XXVI	soft maple (*Acer rubrum*)	tangential, radial	USA: Pennsylvania
LTLSM-III	soft maple (*Acer* spp.)	tangential, radial	USA: Indiana

CHAPTER 6: SUMMARY – NEW CHARACTERS FOR SELECTING BASSOON RESONANT WOODS, A PRELIMINARY LIST OF POTENTIAL NORTH AMERICAN WOODS, AND IDEAS FOR FUTURE STUDY

Bassoons have been made from a limited number of tree species for the last 300 years. During the early development of the instrument, the bassoon was constructed from pear (*Pyrus* spp.) and European maple (*Acer pseudoplatanus*) (Zadro 1975). In the 17th century, the design and, consequentially, the wood choice split in two – the French and German systems (Langwill 1971). The French bassoon's design was kept closer to the older versions of the instrument, in order to keep the unique timbre; however, the wood choice, African blackwood (*Dalbergia melanoxylon*) deviated from tradition (Pitt 1978). The German design was more advanced, becoming larger and adding keys for tonal stability; the European maple (*Acer pseudoplatanus*) was still used for these instruments, and the North American maples (*A. nigrum*, *A. rubrum*, *A. saccharum*, and *A. saccharinum*) were also used (Heckel and Heckel 1931). The manufacturers chose these woods because they all have the same resonant characteristics.

Potential North American Woods

Manufacturers developed a list of ideal characters to describe the bassoon resonant woods through 300 years of trial and error. Because bassoons are created using a lathe, the wood has to be defect-free and straight. While the parts of a bassoon are being shaped, they are being tested for dampening – the muting of a sound. Curving grain is usually blamed for this occurrence, although a study of this for the bassoon has not been done. Schleske (1990) did a similar study on the curving of a violin soundboard's arch that confirmed the role of curving on dampening. Since these are not quantifiable characters, physicists and engineers have tried to find some quantitative measurements to describe the resonant woods. Bucur (2006) listed density and elasticity as good characters for indentifying the resonant woods. There are also logistics to consider. Trees being harvested now for the bassoon have to have a girth (trunk diameter) of at least 18 inches (0.5 meters) in order for the turning blanks to have straight growth rings (Thompson Maple Products, *pers. comm.*). The number of species can be limited by using a minimum trunk diameter of 0.5 meters, a minimum relative density of 0.47, the ease of the wood to be worked, and the clearness of the wood (Table 1).

New Characters

These characters are not fool-proof for determining the bassoon resonant woods. Fox Products Corporation used these characters to find an alternative wood for their bassoons – black walnut (*Juglans nigra*). A black walnut bassoon was built and tested in the workroom and concert hall. The instrument played in the workroom, but its sound could not project to the back of the concert hall (Owen, *pers. comm.*). The lack of projection for the black walnut bassoon and the dampening power of a curving grain suggested that anatomy was important. In Chapter 3 axial parenchyma width and the size of the vessels and fibers were determined to be good characters with which to differentiate black walnut (*Juglans nigra*) and the bassoon resonant woods. These are not commonly measured. Databases such as Inside Wood have ranges for some of these features, but for a more accurate assessment the species would have to be individually investigated. Using the values found for black walnut (*Juglans nigra*) as an upper limit for viable resonant woods, a table of characters for describing bassoon resonant woods can be made (Table 2). Each of these characters can be used as a comparison point for any potential resonant woods.

Data from tapping and the cell wall would be harder to gather in the field or a workroom. Even though the studies from Chapter 4 confirmed that the variables measured can be used to distinguish bassoon resonant woods from the non-resonant black walnut (*Juglans nigra*), the data cannot be obtained without a full lab set-up, which would not be cost effective. Also, even if a species is resonant, not all the timber from the species will be of equal quality, and cell walls can be affected by the environment (Antonova and Stasova 1993, Antonova and Stasova 1997, Mellerowicz, *et al.* 2001). However, tapping can still be used after a species has been deemed resonant with enough practice. During the study from Chapter 4, sound resulting from tapping black walnut was found to be more muted than that from the resonant woods, but the effect was subtle for some of the specimens and easily noticeable for others.

Until a bassoon is made either from a wood either fitting the new description from Table 2 or one that does not fit, the description put forth remains theoretical. Even though black walnut was found to be unsuitable by Fox, it was only one attempt. Other representatives from different parts of the species range may create a usable bassoon. This aspect was not fully explored in this study, and wood is nothing if not variable.

Ideas for Future Study

In order to take the lists made from theoretical to practical, two things need to happen. Anatomical data needs to be gathered from the species on the list, using cores or chips gathered from trees across the species range. This will help complete the list of bassoon resonance woods. Afterwards, bassoons, or at least one of the bassoon joints, need to be made from species on both the suitable woods list and those woods discarded by anatomy. The bassoons would be a practical test of the importance of the anatomical characters and the viability of using these as a description of resonance.

The cell wall in this study was not examined as closely as initially planned. It is an important feature, because it affects all the mechanical properties of wood. There is not much work comparing the cell walls of each cell type, and it could provide important insight to how each face interacts with vibration. Atomic force microscopy, transmission electron microscopy, and fluorescence microscopy should provide important information.

Another avenue of study involves the curving growth ring dilemma mentioned in chapters 1 and 3. If the discarded joints can be compared to a joint that made it all the way through the shaping process, the maximum angle a growth ring can curve without causing any dampening effect could be found. Another method would be to recreate Schleske's experiment, modifying it to fit to the parameters used by the bassoon. Coupling these ideas with an anatomical approach should provide insights to why the radial face seems to be the preferred resonance face, by exploring the proportion of merging faces in relation to dampening.

Even though the material from which bassoons are made do not affect timbre, it does work in more subtle ways, which is just as important for making music as the timbre. This study was a starting point, and much more needs to be done to fully understand wood's affect on the bassoon.

Table 1 List of North American hardwoods (angiosperms) that have minimum trunk diameter of 0.5 m, a minimum relative density of 0.45, are easy to work, and have clear wood (United States Forest Products Laboratory 1974). The species studied for the dissertation are in bold.

Species	Common name	SG	Trunk diameter (m)
Acer nigrum	**black maple**	**0.57**	**1**
Acer rubrum	**red maple**	**0.54**	**0.8**
Acer saccharinum	**silver maple**	**0.47**	**1.2**
Acer saccharum	**sugar maple**	**0.63**	**1**
Arbutus menziesii	Pacific madrone	0.69	0.9
Kalmia latifolia	Mountain laurel	0.68	0.6
Liquidambar styraciflua	Sweetgum	0.52	1
Oxydendrum arboreum	Sourwood	0.55	0.6
Prunus serotina	Black cherry	0.5	1.5
Castanopsis chrysophylla	Giant chinkapin	0.46	1.2
Prosopis glandulosa	honey mesquite	0.82	1.2
Magnolia grandiflora	southern magnolia	0.5	1
Ailanthus altissima	Tree-of-heaven	0.53	0.6
Betula alleghaniensis	yellow birch	0.62	0.6
Betula lenta	sweet birch	0.65	1.5
Betula papyrifera	paper birch	0.55	0.6
Carpinus caroliniana	American hornbeam	0.7	0.6
Celtis occidentalis	Hackberry	0.56	1.2
Cornus florida	Flowering dogwood	0.8	1
Diospyros virginiana	Persimmon	0.78	0.6
Fagus grandifolia	American beech	0.64	1.2
Fraxinus latifolia	Oregon ash	0.55	0.6
Fraxinus nigra	black ash	0.49	0.6
Gleditsia triacanthos	Honeylocust	0.67	0.9
Gymnocladus dioicus	Kentucky coffeetree	0.6	1
Ilex opaca	Holly	0.57	0.6
Juglans nigra	**black walnut**	**0.55**	**1**
Lithocarpus densiflorus	Tanoak	0.75	0.6
Maclura pomifera	Osage orange	0.85	1
Magnolia acuminata	cucumbertree	0.48	1.2
Magnolia virginiana	Sweetbay	0.48	1

Table 1 continued

Species	Common name	SG	Trunk diameter (m)
Nyssa aquatica	water tupelo	0.5	0.6
Nyssa sylvatica	black tupelo	0.5	1.2
Ostrya virginiana	Hophornbeam	0.7	0.6
Platanus occidentalis	Sycamore	0.49	1
Robinia pseudoacacia	Black locust	0.69	1
Sassafras albidum	Sassafras	0.46	1.5
Umbellularia californica	California laurel	0.55	1
Quercus coccinea	scarlet oak	0.67	1
Quercus falcata	southern red oak	0.52	1
Quercus falcata var. *pagodifolia*	cherrybark oak	0.68	1
Quercus nigra	water oak	0.63	0.6
Quercus palustris	pin oak	0.63	0.6
Quercus phellos	willow oak	0.69	1
Quercus rubra	northern red oak	0.63	1
Quercus velutina	black oak	0.61	1.2
Ulmus alata	winged elm	0.66	0.6
Ulmus americana	American elm	0.5	1.5
Ulmus crassifolia	cedar elm	0.64	1
Ulmus rubra	slippery elm	0.53	1
Ulmus thomasii	rock elm	0.63	0.6

Table 2 Characters and values that can be used to define a bassoon resonant wood.

Character	Value
trunk diameter	$\geq$ 0.5 m
growth ring curvature	straight
clarity of wood	clear
twisting of wood along vertical axis of growth	none
defects	none
axial parenchyma width	< 10 μm
fiber length	<1000 μm
vessel width	< 100 μm
vessel length	< 200 μm
relative density (specific gravity)	$\geq$ 0.45

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